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Investigating the potential role of Akkermansia muciniphila supplementation in neuroinflammation: A progress report

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Investigating the potential role of *Akkermansia muciniphila* supplementation in neuroinflammation: A progress report

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HPI-CoBRE

Introduction

Gut bacteria are important for proper development and function of the host immune system (1). Recent studies have provided association of altered microbiome in both food allergy and neuropsychiatric disorders (2, 3) indicating potential role of microbiome in regulating Gut-Brain-Axis. ***Akkermansia muciniphila*** belongs to the phylum Verrucomicrobia, is a commensal mucin degrading bacterial species (4). ***A. muciniphila*** protects the gut barrier by facilitating host mucus production. Since patients with food allergy have increased gut permeability, protection of intestinal barrier by increased mucus production may be beneficial in preventing allergen and pathogen infiltrations, hence, minimizing inflammation. ***A. muciniphila*** is well known in preventing obesity and diabetes but its role in food allergy and neuropsychiatric disorders has yet be ascertained.

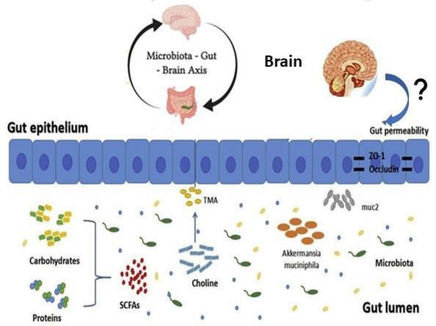

Fig.1. The role of *A. muciniphila* in Gut-Brain Axis

Background

Our previous study used mouse model of Cow milk allergy (CMA) involving mice sensitized to a bovine whey allergen, with β-lactoglobulin (BLG: Bos d 5) to investigate food allergy induced changes in brain pathophysiology and behavior (5, 6). A peculiar shift in microbiota was observed. BLG-sensitized male mice with altered behavior showed a marked decrease in ***A. muciniphila*** population (7, 8).

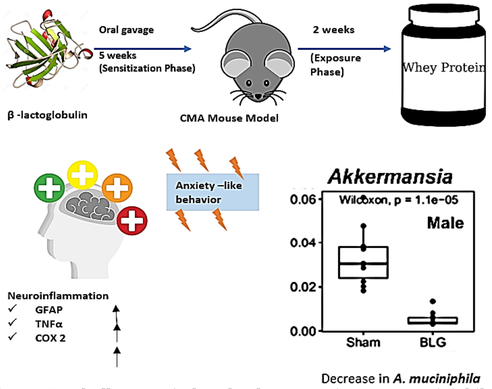

Fig.2. Food-allergen induced decrease in *A. muciniphila* and neuroinflammation

Hypothesis and Objectives

Based on the findings from our earlier studies, hypothesis was proposed. BLG sensitized intestinal immune cells favors decrease in the ***A. muciniphila*** population which may cause breakdown of intestinal barrier. This may further allow translocation of pathogens and allergens causing changes in immune responses of the host brain. Thus, the present study aims at investigating the potential role of ***A. muciniphila*** supplementation in restoring gut microbial population and neuro-inflammation .

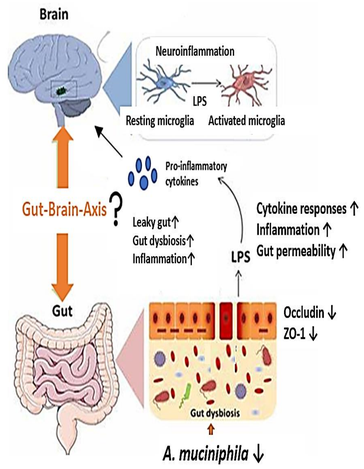

Fig.3. Proposed hypothesis showing effects of *A. muciniphila* reduction in alteration of brain pathophysiology

Methods

1. Experimental design. C57BL/6J male mice were sensitized to BLG by oral gavage for 5 weeks and placed on the whey-diet for 2 weeks. Upon confirming ***A. muciniphila*** loss, daily oral supplementation ($2x10^8$ cfu/200 µL, ***A. muciniphila*** live) was given for 4 weeks. Heat killed ***A. muciniphila*** and PBS was used as a negative control.

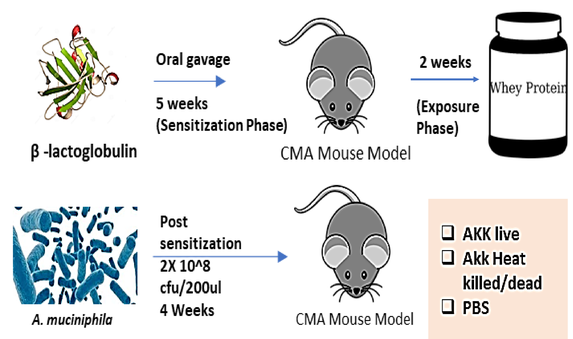

Fig.4 Experimental Layout showing the BLG sensitization and *A. muciniphila* supplementation

Methods (Cont'd)

2. Biological sample collection. Mice will be euthanized via CO2 asphyxiation. Atrial blood samples will be collected, and the brains will be dissected after intracardiac perfusion with PBS. The brain will be bisected sagittally, and the left hemisphere will be fixed in 4% PFA for histological analyses, while the right hemisphere will be microdissected into brain regions. These samples will be assayed for inflammatory cytokines and chemokines.

3. Immunohistochemistry. Sections from the brains (40 µm) has been stained immunostained for glial fibrillary acidic protein (GFAP) (1:1000), CD45 (1:500), Iba-1 (1:1000), and IgG extravasation (1:500). Black Gold II myelin staining has been done for myelin basic protein (MBP) or proteolipid protein (PLP). Sections from ileum (10 µm) will be stained for occludin (anti-mouse occludin, 1:100, Invitrogen) or 5-hmc (1:8,000, Active Motif). Immunoreactivity was visualized with Vector Elite ABC kit with VIP as the chromogen. Densitometry was performed using Adobe Photoshop.

4. **16S rRNA gene sequencing.** Faecal samples has been subjected to 16S rRNA gene sequencing to detect any changes in the gut microbiota. The abundance of *A. muciniphila* will be measured using qPCR with a specific primer set (7), S-St-Muc-1129-a-a-20 and S-St-Muc-1437-a-A-20.

Results

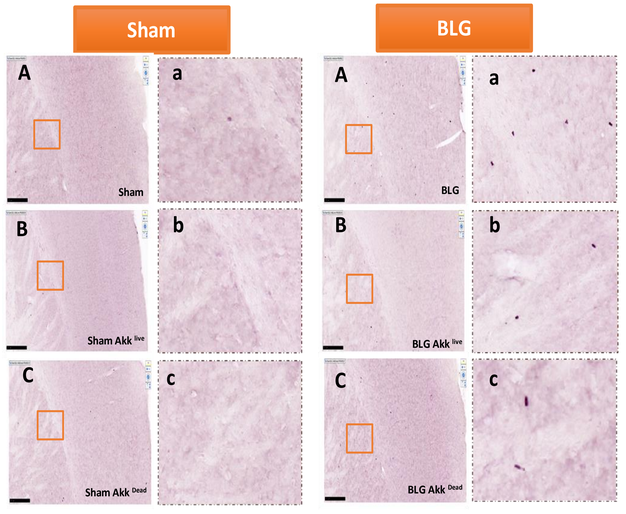

Fig.5. CD45 immunoreactivity was observed in the thalamus of brain in BLG sensitized mice (A: Sham/BLG; B:Sham/ BLG AKK ^{live}; C: Sham/ BLG AKK ^{dead} ;20x objective). Magnified images (a, b, c) of the same section shown at the right.

Discussion

❖CMA-activated host immune cells cause microbiota shifts and a peculiar reduction *A. muciniphila* population associated with significant anxiety-like behavior.

➢**BLG sensitization increases the CD45 immunopositivity**. These changes seems to support the hypothesis. However, the changes needs to be quantified and analyzed statistically.

❖Immunostaining with antibodies against GFAP, IgG extravasation, Iba-1 has been performed to assess astrogliosis, BBB permeability and microgliosis, respectively. **Black Gold II myelin staining** has been performed to observe any changes in myelination.

➢These studies are on-going and under qualitative analysis.

❖**16S rRNA gene sequencing** data is under observation.

Expected Outcomes/Conclusions

❖We expect that the *A. muciniphila* supplementation will reduce the CMA-induced brain pathophysiology, proinflammatory cytokine and chemokine levels, and anxiety-like behavior.

➢*A. muciniphila* facilitates host mucus production and hence, may restore intestinal barrier integrity.

❖It may not fully colonize, but recovery of mucus thickness and MUC2 production, as well as short-chain fatty acids (SCFA) levels will be considered as its beneficial effects (9).

➢*A. muciniphila* stimulates mucus turnover rate by making SCFA from the degraded mucin, the preferable energy sources for the host epithelium which synthesize and secret mucin.

➢The *A. muciniphila* supplementation is known to increase the number of mucin-producing goblet cells in mice (10).

References

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