Inflammation and dysbiosis in the intestinal-specific *Cftr* knockout mouse

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Background

- Increasing evidence supports the association between intestinal microbial dysbiosis and inflammation in individuals affected by inflammatory bowel diseases (IBD).
- The global knockout of *Cftr* in mice as a model of cystic fibrosis shows intestinal dysbiosis and inflammation.
- Since leukocytes express CFTR, we asked whether the intestinal-specific *Cftr* KO (i*Cftr* KO) mice also show dysbiosis and bowel inflammation.
- Previously, we found that i*Cftr* KO mice have decreased fecal microbial diversity and a population with potential pathobionts.
- To prevent intestinal impaction in the mice, we transitioned the mice between two impaction preventative diets: osmotic polyethylene glycol (PEG) laxative in drinking water with pellets and drinking water with a complete liquid diet.
- We assessed inflammation by utilizing an ELISA assay specific for fecal calprotectin, a protein predominantly secreted by neutrophils and used as a marker in IBD.

Hypothesis

We hypothesized that there will be an increase in fecal calprotectin concentration in i*Cftr* KO mice fed a complete liquid diet compared to the diet including the PEG laxative.

Methods

Experimental Groups

- Intestinal-specific *Cftr* KO mice [B6.Cg-Tg(Vil1-cre)-Cftrf10/f10] and their sex-matched wild-type littermates (WT)

Dietary Intervention and Feces Collection

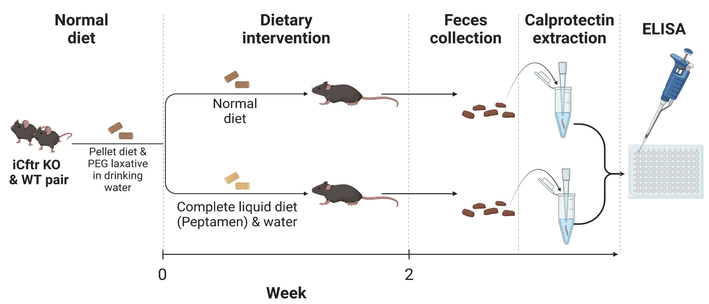

Figure 1. Dietary intervention and feces collection for calprotectin extraction used for ELISA assay. We transitioned sex-matched pairs of i*Cftr* KO and their WT littermates from a normal diet consisting of pellets and PEG laxative in their drinking water (Colyte) to a complete liquid diet (Peptamen®) with water. After two weeks of the diet intervention, we collected the fecal samples. Utilizing the fecal calprotectin ELISA protocol, we extracted the calprotectin from the samples.

Calprotectin Sandwich ELISA Protocol

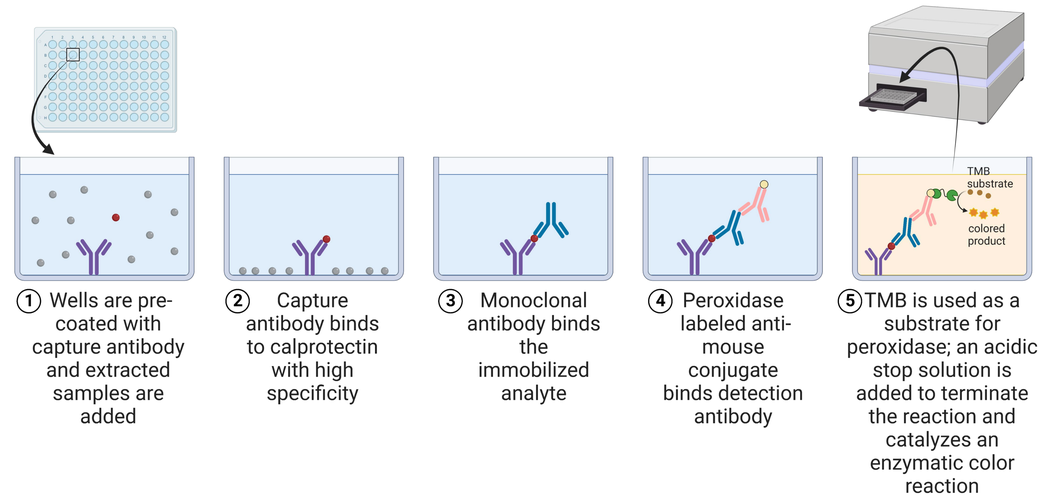

Figure 2. Fecal calprotectin sandwich ELISA assay. Following step 5, we transferred the 96-well plate containing samples, controls, and standards to a spectrophotometer plate reader to measure absorbances.

Results

Fecal Calprotectin in Colyte-treated and Peptamen®-fed Mice

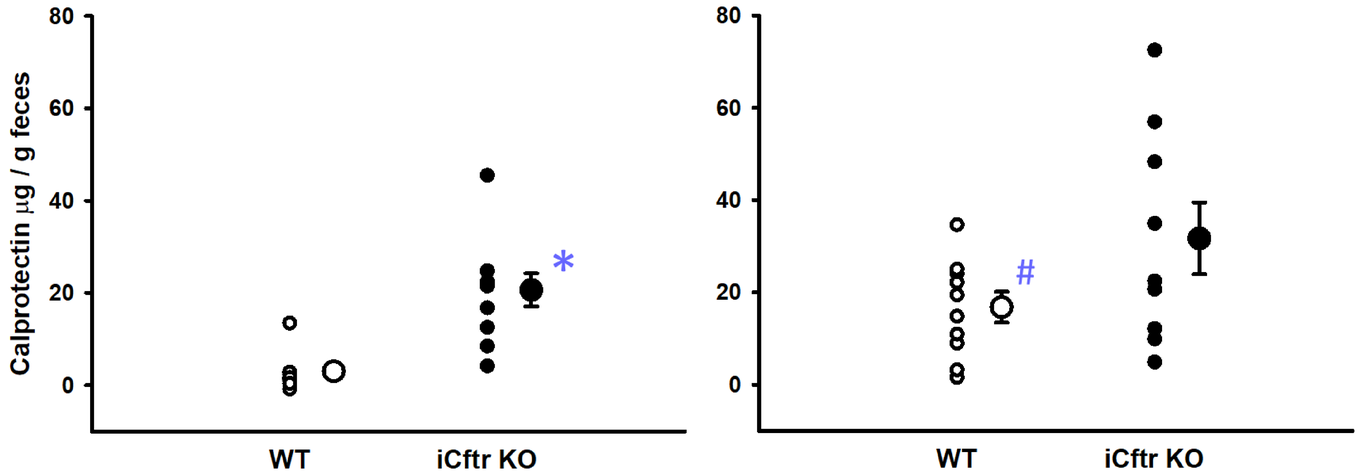

Figure 3. Calprotectin in Colyte-treated and Peptamen®-fed mice. Represented in the Colyte figure (left), the i*Cftr* KO Colyte-fed mice show a significant increase of fecal calprotectin concentration versus WT Colyte-fed mice. (*) p< 0.01 using Mann-Whitney Rank Sum Test for i*Cftr* KO Colyte vs WT Colyte (n= 10 WT/i*Cftr* KO pairs). In the Peptamen figure (right), the WT Peptamen®-fed mice show a significant increase in concentration of fecal calprotectin versus the WT Colyte-fed mice. (#) p= 0.002 using a Welch's t-test for WT Peptamen® vs WT Colyte (n= 10 WT, 9 i*Cftr* mice).

Conclusions

- The intestinal knockout of *Cftr* is sufficient to induce inflammation.
- The i*Cftr* KO mice have intestinal inflammation regardless of whether they are Colyte-treated or Peptamen®-fed.
- Going forward, we will run more Peptamen® fecal samples for the calprotectin ELISA assay.
- Additionally, we are examining the composition of the fecal microbiome of Colyte-treated and Peptamen®-fed mice.

Acknowledgements

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