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 (iCftr KO) mice also show dysbiosis and bowel inflammation.
- Previously, we found that iCftr 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 iCftr 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

- Normal diet
- Dietary intervention
- Feces collection
- Calprotectin extraction
- ELISA

Figure 1. Dietary intervention and feces collection for calprotectin extraction used for ELISA assay. We transitioned sex-matched pairs of iCftr 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

1. Wells are precoated with capture antibody and extracted samples are added
2. Capture antibody binds to calprotectin with high specificity
3. Monoclonal antibody binds the immobilized analyte
4. Peroxidase labeled antimouse conjugate binds detection antibody
5. TMB is used as a substrate for peroxidase; an acidic stop solution is added to terminate the reaction and catalyzes an enzymatic color reaction

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 iCftr 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 iCftr KO Colyte vs WT Colyte (n= 10 WT/iCftr 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 iCftr mice).

Conclusions

- The intestinal knockout of Cftr is sufficient to induce inflammation.
- The iCftr 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.

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