CFTR Loss of Function in Innate Immune Cells Does Not Affect Intestinal Movement

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Introduction

Cystic Fibrosis (CF) is a chronic, progressive and debilitating genetic disorder, caused by mutations in the CF transmembrane-conductance regulator gene (cfrtr), which encodes for the CFTR protein, a cAMP-activated chloride channel. The CFTR channel plays a critical role in regulating chloride and bicarbonate passage into and out of cell membranes. CF is a multi-system disease that affects mostly the lungs, but also the pancreas, liver, kidneys, and intestine. Long-term issues include difficulty breathing and coughing up mucus as a result of frequent lung infections. Other signs and symptoms may include sinus infections, poor growth, fatty stool, clubbing of the fingers and toes, and infertility in most males. CF currently has no cure, but treatments have been developed to increase the quality of life and life expectancy of those with the condition. Prior to the invention of treatments for CF, children suffering the disease rarely lived past 5, but now CF patients can expect to make it to their mid-40s. While pulmonary disease is the leading cause of death in adult patients, intestinal disease claims the early morbidity and mortality in those with CF. The intestinal disease can be characterized by small intestinal bacterial overgrowth, large intestine dysbiosis, and intestinal inflammation and obstruction. CF has long been considered an epithelial disease. However, previous research has shown that the immune system plays a major role in the symptoms of CF. The role of innate immune cells in CF is not very well understood. This project seeks to identify a relationship between defects in certain innate immune cells caused by CF and intestinal passage times, an indicator of the severity of the symptoms of CF.

Methods and Materials

- Mice from 4 different lines of CFTR knockout (KO), including myeloid-CFTR-KO (Mye-CF), neutrophil-CFTR-KO (Neu-CF), macrophage/monocyte-only CFTR-KO (Mac-CF), and Pan-CFTR-KO (Pan-CF), were each co-housed with their sibling CFTR Exon-10 floxed mice (WT). Each group of Mye-CF, Neu-CF and Pan-CF consisted of 6 mice with 3 of each sex, for a total of 18 CF knockout mice and 18 respective WT mice, with an equal number of males and females for each genotype. However, the Mac-CF line of mice only consisted of 3 male mice due to a lack of resources.
- Cell genotyping was performed on each mouse. A DNA sample was obtained from a small segment of tail by clipping, followed by lysis with 50 µl of sodium hydroxide. Then PCR was run on the lysed DNA samples and the amplicons were resolved in gel electrophoresis. The results of the gel electrophoresis were then analyzed in order to confirm the genotype of each mouse.
- Carmine red (6% w/v) and methyl cellulose (0.5% w/v) were dissolved in sterile hot drinking water and each mouse was given an oral gavage of the solution based on how much each mouse weighed (10 µl per gram).
- The data was gathered from each mouse, the time of intestinal transit for each mouse was calculated, and the data were analyzed using Student’s t-test.

Results

4 different lines of CFTR KO mice and their sibling WT mice are submitted for testing

The genotype of each mouse is confirmed with PCR

Each mouse is given an oral gavage of carmine red dye, and the time is recorded

The data produced is analyzed to determine for statistical significance

The time of the first completely red fecal pellet produced by each mouse is recorded

Genotyping Data

Fig. 1. Representative data of genotyping for CF mice, and their WT siblings through PCR and gel electrophoresis.

Confirmation of CFTR KO

Fig. 2. PCR validation of CFTR KO in PMN (neutrophils) and Mono (Monocytes/macrophages) FACS-sorted from the peripheral blood of Cfrtr10 and Mye-CF. Tail samples were served as a control.

Conclusion

- There was a statistically significant relationship in intestinal transit times between Pan-CF mice and their WT siblings.
- There was no statistically significant relationship between CFTR KO in innate immune cells and their WT siblings.
- The effect of CFTR KO in innate immune cells on intestinal transit time in mice is either limited or synergized with epithelial cells.
- The CFTR defect in epithelial cells could lead to slower intestinal transit times due to decreased peristalsis, increased mucus in the intestines, and intestinal obstruction.
- A limiting factor for this experiment was the sample sizes of the mice. This was especially apparent in the Mac-CF mice, as the sample size of the Mac-CF mice was only 3, just enough to test for statistical significance. As the sample size n increases, the chance for statistical significance between datasets is increased.

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