

Targeted epithelial disruption impacts colonic mucus and microbiota in mice

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Background and aims: The intestinal barrier involves in its luminal side three tightly connected partners: microbiota, mucus, and epithelium. To study their interactions, we used here a model of mice that present a targeted epithelial disruption, consequence of the expression of a transgene encoding CA-MLCK (Constitutively Active Myosin Light Chain Kinase), and we determined mucus and microbiota modifications in these animals.

Methods: Eight to ten-weeks old female CA-MLCK transgenic mice and their control C57Bl6 WT littermates were used in this study. Whole gut intestinal permeability of CA-MLCK and control mice (n=8-12 per group) was determined by measuring plasma fluorescence three hours after fluorescein sodium salt gavage (MW 376) and normalized with animal weight (ng/mL of plasma/g of BW). Properties of colonic mucus were determined as follows: (i) thickness of the mucus inner layer attached to the epithelium, and goblet cells number of CA-MLCK and control mice (8-12 animals per group), were evaluated by PAS/alcian blue staining, (ii) mucin O-glycosylation from scrapped mucus was analyzed by mass spectrometry. Colonic content was harvested from transgenic and control mice (n=16 per group). Eighteen gut bacterial communities from colonic content were monitored by qPCR using GUt Low Density Array method. Comparisons of permeability and mucus properties were performed by unpaired t-test whereas relative abundance of bacterial communities was analyzed using Mann-Whitney test followed by the Benjamini-Hochberg adjustment of p- values for multiple comparisons (q<0.05).

Results: CA-MLCK mice showed elevated gut permeability (31.8 ng/mL/g±4.2 vs. 18.9±1.9, p=0.01) without any difference in body weight. The thickness of colonic mucus inner layer was significantly reduced in CA-MLCK mice compared with controls (10.7 µm±0.9 vs. 13.0±0.7, p=0.05) while the percentage of goblet cells within the epithelium remained unchanged (60%±4 vs. 62±2, p=0.76). Substantial changes in mucin O-glycosylation were observed, with decrease in sialylated structures. Regarding microbiota, we observed a general increase in Firmicutes and a decrease in Bacteroidetes in CA-MLCK mice compared to controls. Specifically, the relative abundance of *Clostridium* cluster XIVa, belonging to Firmicutes was increased in CA-MLCK mice (q=0.025).

Conclusion: Subtle alterations of the epithelial barrier trigger changes in both mucus and microbiota in colon. Inner mucus layer thickness and mucin O-glycosylation were affected, while goblet cells number remained unchanged. Microbiota analysis revealed an increased abundance of *Clostridium* cluster XIVa. This cluster is known to encompass bacteria able to produce butyrate and colonize mucus. These findings support the CA-MLCK mice as a relevant model for studying the mechanistic basis of the cross-talk between epithelium, mucus and microbiota.