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Effect of Basal Diet and Black Raspberry Supplementation on Gene Biomarkers of "Leaky Gut" in Mouse Model of Colitis-Associated Colorectal Cancer

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Effect of Basal Diet and Black Raspberry Supplementation on Gene Biomarkers of "Leaky Gut" in Mouse Model of Colitis-Associated Colorectal Cancer

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Background

- Colorectal cancer is currently the third leading cause of cancer-related deaths in the world, with the risk of colorectal cancer increasing in individuals who suffer from colitis, inflammation of the colon lining, seen in Irritable Bowel Disease.
- "Leaky gut" is a term used to describe increased intestinal permeability and is closely linked to gut inflammation. The development of leaky gut is associated with dysbiosis of the gut microbiota, which is caused by diet. This dysbiosis creates pro-longed inflammation and affects genes involved in intestinal integrity.
- Previous studies completed by our group have demonstrated that the Total Western Diet has a promoting effect on colitis-associated colorectal cancer (CAC) in mice leading to markedly increased colon inflammation as compared to mice consuming a healthy diet.
- Black raspberries (BRB) are a whole food that contain anti-inflammatory bioactives that have been shown to have a protective effect on the colon epithelium and can influence the gut microbiome.

Objective

The objective of this study is to determine how basal diet and supplementation with BRB affects expression of genes involved in intestinal integrity and permeability before, during, and after colitis.

Hypothesis

We hypothesize that expression of genes critical for maintaining the gut barrier and responding to bacterial infiltration will be differentially expressed in mice fed the TWD as compared to the AIN healthy diet, and their expression restored when supplemented with BRB.

Methods

Sample Collection and RNA Extraction

- Samples of the colon mucosa were taken from mice at the 3 timepoints of interest. RNA was extracted from samples using the RNeasy® Plus Micro Kit from Qiagen.

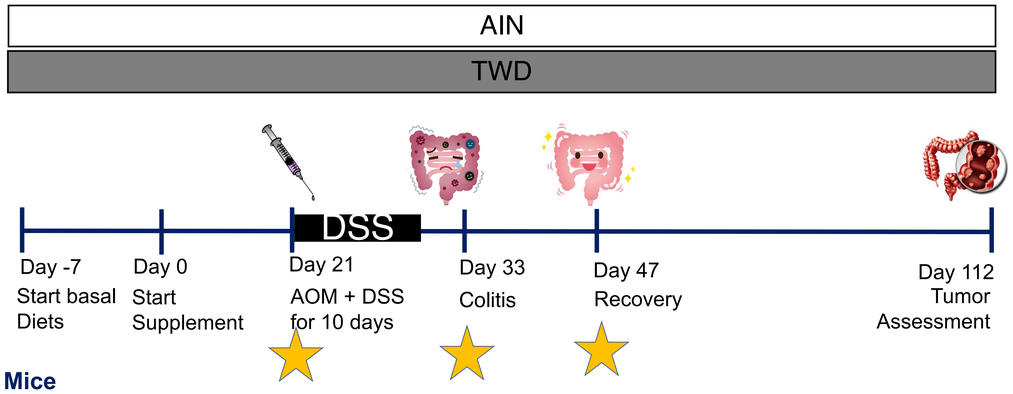
Reverse Transcription and qPCR Reaction

- Extracted RNA was reverse transcribed into cDNA using a SuperScript® IV Reverse Transcription Kit from Thermofisher.
- The cDNA was then amplified using a PowerTrack™ SYBR™ Green Master Mix from Thermofisher to perform a quantitative polymerase chain reaction.

Data Processing

- The resultant threshold (C_t) values from the qPCR reactions were quantitated using the delta-delta C_t method to compare gene expression between groups.

Study Design



Mice

- C57BL6/J mice
- 263 Males and 272 Females
- 5 weeks of age

Experimental diets

- **AIN93G:** control diet that promotes rodent health
- **Total Western Diet (TWD)**: promotes inflammation-associated colorectal carcinogenesis

Inflammation and Cancer Model

- 10 mg/kg azoxymethane (AOM) to initiate carcinogenesis on day 14 + 1% (w/v) dextran sodium sulfate to promote colon tumor development for 10 days
- During the colitis portion of the study, the mice experience symptoms of leaky gut, including diarrhea and bloody feces.

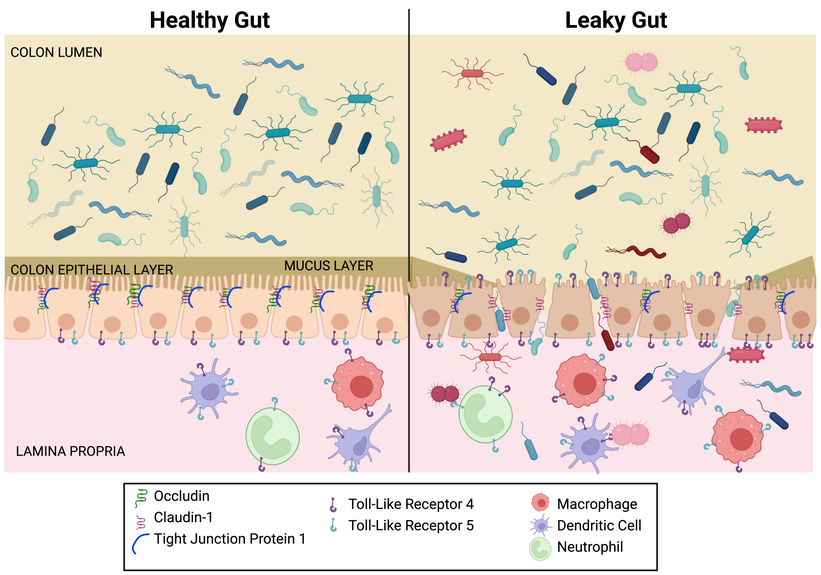
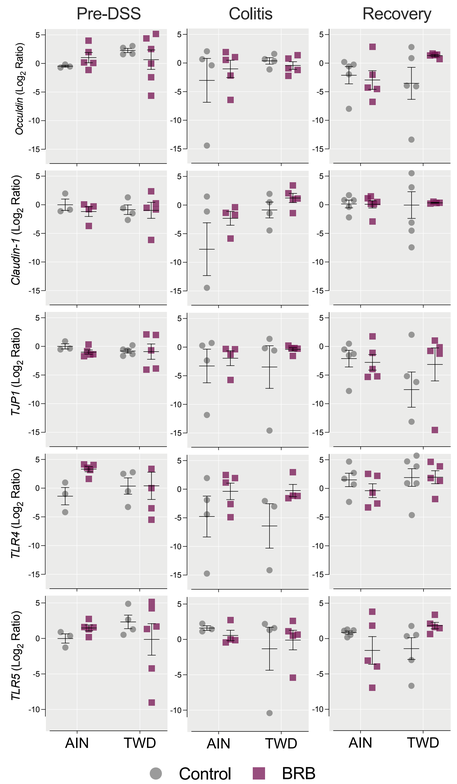


Figure 1. Contrast between a healthy gut and a leaky gut. In the healthy gut, the microbiome is in homeostasis and there is an intact layer of epithelial cells with robust tight junction connections. These tight junctions allow for selective passage of material through the epithelium while preventing pathogens from getting through. In the leaky gut, the microbiome is in dysbiosis, and pathogenic bacteria are present. Tight junctions are disrupted resulting in openings in the epithelium, through which translocation of bacteria and Toll-Like Receptors occur. This results in activation of immune and inflammatory signaling via immune cells.

Preliminary Data



Occludin
- Involved in tight junctions between epithelial cells
- Decreased expression in leaky gut

Claudin-1
- Involved in tight junction and inflammatory pathways
- Increased expression in leaky gut, contributes to sustained inflammation

Tight Junction Protein 1
- Involved in tight junctions between epithelial cells
- Decreased expression in leaky gut

Toll-Like Receptor 4
- Involved in pathogen recognition, mediates inflammatory response
- Increased expression in leaky gut

Toll-Like Receptor 5
- Involved in pathogen recognition, mediates inflammatory response
- Increased expression in leaky gut

Figure 2. Expression of gene biomarkers in the colon mucosa obtained at pre-DSS, colitis, and recovery timepoints in mice fed either AIN or TWD and supplemented with BRB. The values in these plots are represented as log base 2 ratios with respect to GAPDH as a housekeeping gene. Statistical analysis is forthcoming.

Discussion

Based on initial assessment of preliminary data, we did not observe strong responses for any of the examined genes for either basal diet nor black raspberry intervention. However, we do note the surprising high level of variability in these data, which were also noted for other measures (colitis symptoms and measures of microbial diversity, data not shown). Statistical analyses are pending, as is further optimization of the qPCR protocol for mucosal RNA samples obtained from mice exposed to DSS, an agent that may interfere with PCR reagents.

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