Fecal microbiota transfer (FMT) from tumor-bearing mice fed the total Western diet (TWD) does not contribute to a higher tumor burden in mice fed a healthy diet

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Background

- Colorectal cancer (CRC) is the third most common cause of cancer death in the United States. Diet and gut microbiome have both shown to influence the development of colitis associated colorectal cancer (CAC).
- The total western diet (TWD) has been shown to increase colitis and tumorigenesis but the connection to the microbiome composition is less clear.

Hypotheses

1. In mice fed a standard healthy diet, FMT from mice fed the pro-inflammatory TWD diet will exacerbate inflammation and tumorigenesis as compared to FMT from mice fed the healthy AIN diet.
2. Conversely, for mice fed the Western diet directly, FMT from mice fed the healthy AIN diet will confer some protection leading to reduced inflammation and tumorigenesis as compared to FMT from mice fed the TWD.

Methods

- Single treatment of a carcinogen and 10 days of induced inflammation was used as a baseline in this cancer model. Variables of diet and microbiome in four groups, using a 2x2 factorial design, relate to correlations in the three measurable severities of colitis, histopathology, and tumor assessments.
- A daily antibiotic cocktail was used to clear the microbiome before daily and then weekly fecal microbiota transfer (FMT) was delivered via oral route from mice fed either AIN or TWD in a previous study.

Acknowledgments

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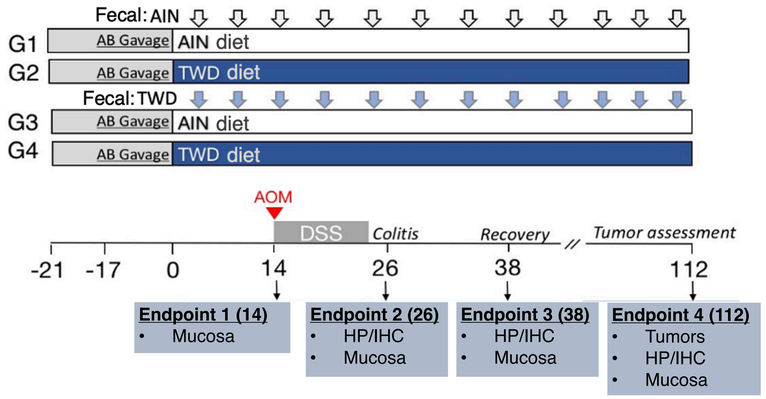
- Utah Agricultural Experiment Station

Study Design



Cancer Model
- 10 mg/kg azyoxymethane (AOM) initiates carcinogenesis
- 1% dextran sodium sulfate (DSS) promotes tumorigenesis (10 days)

Mice
- C57BL6/J
- 320 males at 5 weeks old

AIN93G Diet (AIN)
- control diet that promotes rodent health

Total Western Diet (TWD)
- promotes inflammation-associated colorectal carcinogenesis

Colitis Assessment
- Colitis was scored using the disease activity index (DAI) during active colitis and two weeks later during recovery. The DAI score accounts for apparent blood in the feces, rectal bleeding, stool quality and loss of body weight.

Histopathology Assessment
- Colon tissues were sectioned, H&E stained, and then examined by board certified pathologist who scored blinded samples for evidence of inflammation and mucosal damage.

Tumor Assessment
- At the terminal time point, colon tissues were blinded and the examined by light microscopy to identify tumors, the number of which for each animal was noted (multiplicity) and the size of which was measured by digital caliper and calculated as tumor burden.

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Results

FIG. 1. Disease Activity Index

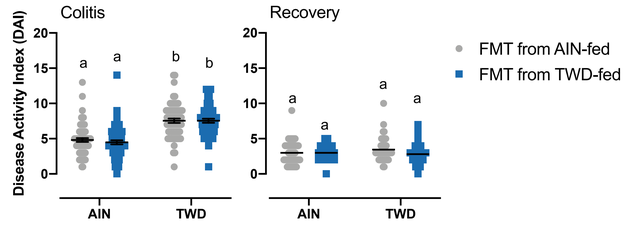

Fig. 1. Disease activity index (DAI) individual values are shown with mean ± SEM (colitis, n=56-65; recovery n=37 to 45). For each timepoint, different letters indicate a significant difference among treatment groups as determined by two-way ANOVA followed by Tukey HSD post-hoc test for multiple comparisons.

FIG. 2. Histopathology of colon mucosa

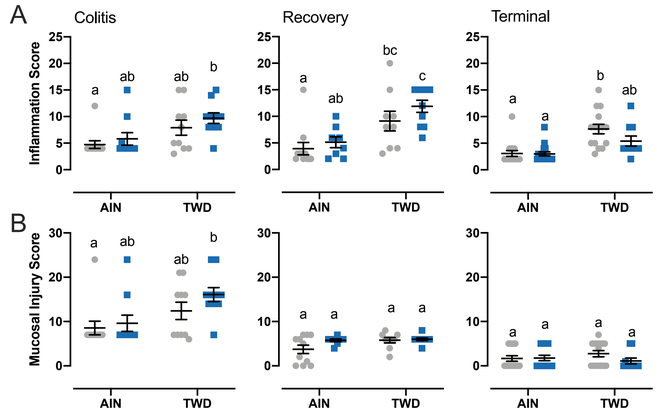

Fig. 2. Histopathology scores of inflammation (A) or mucosal injury (B) across time points are shown as individual values with mean ± SEM (colitis, n = 56 to 65; recovery, n=37 to 45). For each timepoint, different letters indicate a significant difference among treatment groups as determined by two-way ANOVA followed by Tukey HSD post-hoc test for multiple comparisons.

FIG. 3. Colon tumorigenesis

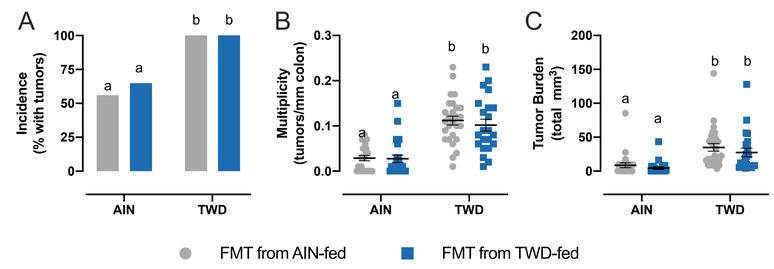

Fig. 3. Tumor incidence (A), multiplicity (B) and burden (C) are shown as individual values with mean ± SEM (for B,C only) (n = 22 to 27). For (A), different letters indicate difference tumor incidence rate as determined by Fisher's exact test. For (B-C), different letters indicate a significant difference among treatment groups as determined by two-way ANOVA followed by Tukey HSD post-hoc test for multiple comparisons.

Preliminary Conclusions

- AOM+DSS-induced colitis as measured by the DAI was higher in mice fed the TWD shortly after DSS treatment, but was not impacted by FMT from TWD-fed mice. No differences in DAI were noted by the recovery time point.
- The histopathology scores for inflammation and mucosa injury and the tumor outcomes followed a similar pattern, with the dominant effect being the diet fed to these experimental mice, rather than FMT from mice previously fed AIN or TWD diets.
- Overall, these preliminary analyses indicate a stronger effect of diet on inflammation-associated colon tumorigenesis than FMT from mice fed the TWD that harbored more severe disease than did those previously fed AIN diet.
- These data do not provide evidence that FMT conferred the host traits, as mice fed AIN diet but provided FMT from animals previously fed TWD did not have worse outcomes.
- Microbiome analyses are forthcoming.