Validation of a Microbiome Dependent Colitis Model via Fecal Microbial Transplant in Germ-Free IL-10 Deficient Mice

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Abstract

Background: Inflammatory Bowel Diseases (IBD) are immune-mediated intestinal tract diseases. Though specific etiologies remain undefined, proposed pathogenic mechanisms include abnormal inflammatory response to the constitutive intestinal microbiome. Mice deficient in IL-10, a critical cytokine for mucosal immune homeostasis, spontaneously develop enterocolitis; phenotypes are microbiome sensitive.

Methods: To establish a microbiome-dependent colitis model with improved construct validity and translational clinical relevance, we assessed colitis development in female Germ-Free IL-10 knockout mice (GF IL-10 KO; Taconic Biosciences) following inoculation with fecal microbial transplant (FMT) from wild-type C57BL/6NTac mice at the Murine Pathogen Free™ (MPF™) health standard and assessed clinical responses to anti-IL-12/23p40 (anti-p40).

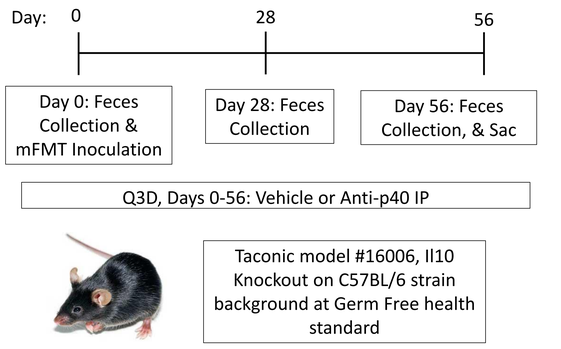


Figure 1: Study Design
Germ-free IL-10 Knockout Mice were inoculated with FMT from wild type C57BL/6NTac mice at the MPF™ health standard on Day 0; a group of mice were maintained germ-free for study duration as naïve controls. FMT-inoculated animals were treated every three days with vehicle or reference compound via intraperitoneal (IP) administration. Animals were assessed for survival and weight loss, as well as presence/severity of diarrhea and bloody stool, on a daily basis. Feces were collected from all animals on Days 28 and 56. At euthanasia on Day 56, colon was excised, measured, rinsed, weighed, and stored for downstream analysis. Feces was processed for ELISA analysis of colitis markers lipocalin 2 (LCN2) and calprotectin. Formalin-fixed colon samples were processed routinely and analyzed for inflammation, gland loss, erosion, and hyperplasia (H&E). Samples were also analyzed for T cells and epithelial proliferation via IHC. Flash-frozen colon samples were processed for multiplex cytokine analysis (Luminex).

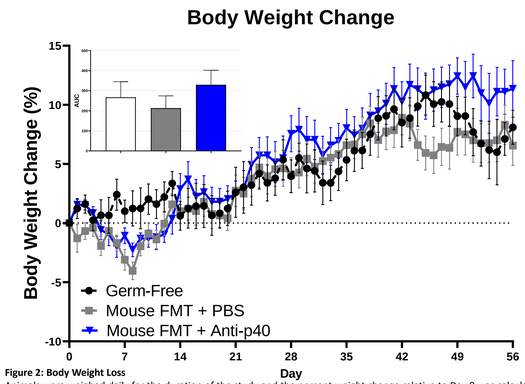


Figure 2: Body Weight Loss
Animals were weighed daily for the duration of the study and the percent weight change relative to Day 0 was calculated. In order to determine overall differences in mean body weight change over time, the area under the curve (AUC) was calculated using the trapezoidal rule transformation and is shown in the figure inset. Mean±SEM is shown. n=6-10 per group

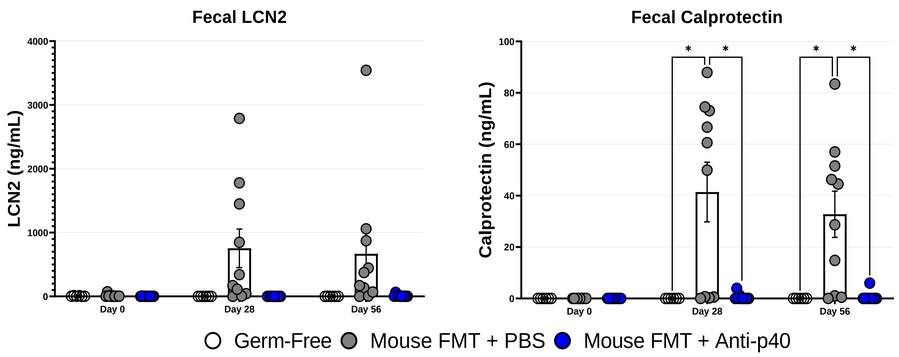


Figure 3: Fecal Lipocalin and Calprotectin
Fecal pellets were collected from individual animals on Days 0, 28, and 56; samples were processed routinely and analyzed by commercially available ELISA for colon inflammation markers lipocalin (LCN2; left) or calprotectin (right). *p<0.05 as determined by 2way ANOVA with Tukey's multiple comparisons test to compare all groups to one another.. Individual values and Mean±SEM are shown. n=6-10 per group

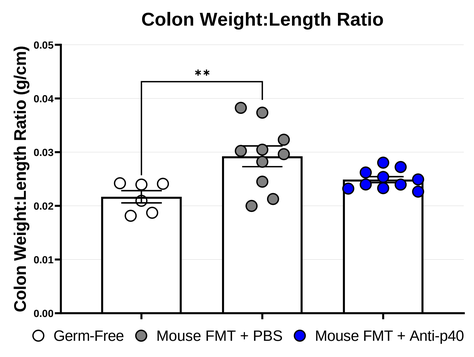


Figure 4: Colon Weight:Length Ratio
After euthanasia on Day 56, the colon was excised, measured, rinsed, and weighed; the colon weight:length ratio was calculated. **p<0.01 as determined by one-way ANOVA with Tukey's multiple comparisons test to compare all groups to one another.. Individual values and Mean±SEM are shown. n=6-10 per group

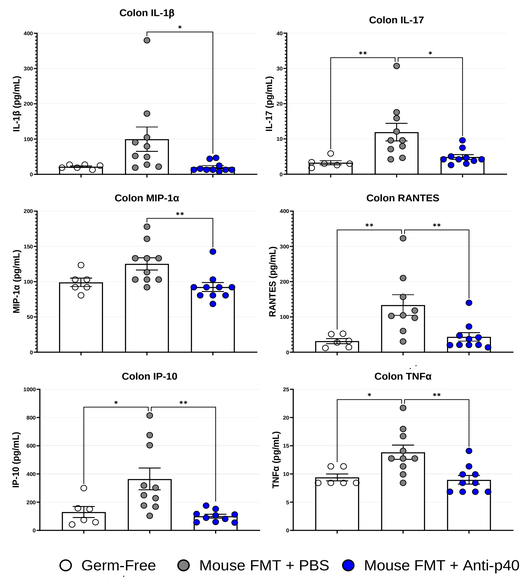


Figure 5: Colon Cytokines
Flash-Frozen colon samples were processed routinely and analyzed by multiplex (Luminex) for panel of cytokines including IL-1β, IL-17, IP-10, MIP-1α, RANTES, and TNFα. *p<0.05; **p<0.01 as determined by one-way ANOVA with Tukey's multiple comparisons test to compare all groups to one another.. Individual values and Mean±SEM are shown. n=6-10 per group

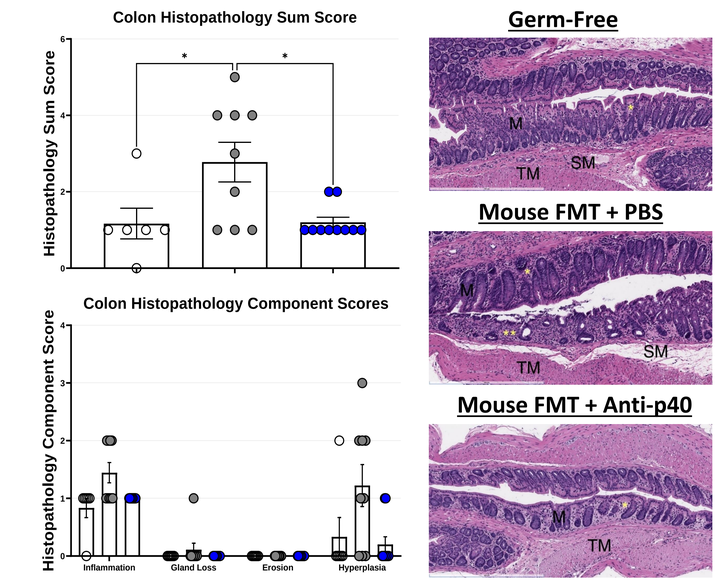
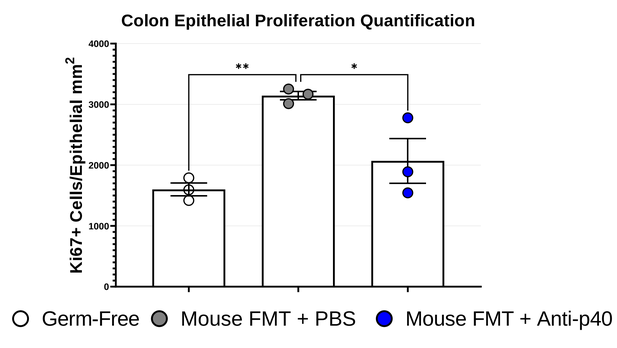


Figure 6: Colon Histopathology
Formalin-fixed colon samples were processed routinely for H&E staining and analysis by an ACVP Board-Certified Veterinary Pathologist. *p<0.05 as determined by non-parametric ANOVA with Dunn's multiple comparisons test to compare all groups to one another. Individual values and Mean±SEM are shown. n=6-10 per group



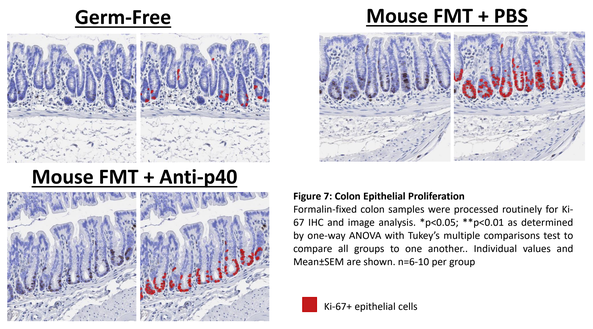
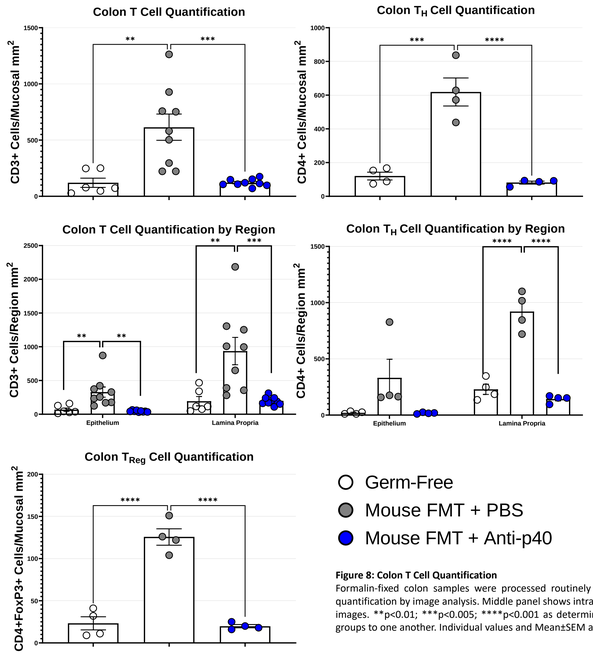


Figure 7: Colon Epithelial Proliferation
Formalin-fixed colon samples were processed routinely for Ki-67 IHC and image analysis. *p<0.05; **p<0.01 as determined by one-way ANOVA with Tukey's multiple comparisons test to compare all groups to one another.. Individual values and Mean±SEM are shown. n=6-10 per group

Ki-67+ epithelial cells



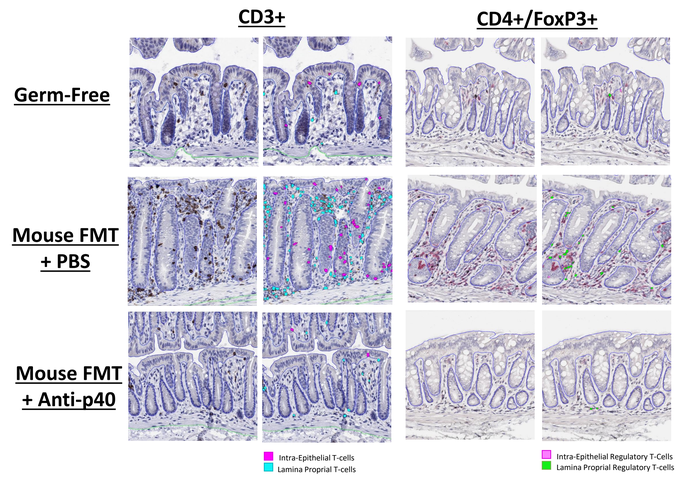


Figure 8: Colon T Cell Quantification
Formalin-fixed colon samples were processed routinely for CD3 (upper left), CD4 (upper right) and CD4+FoxP3 (lower left) IHC and quantification by image analysis. Middle panel shows intraepithelial and lamina propria quantification. Far right panels show representative images. **p<0.01; ***p<0.005; ****p<0.001 as determined by one-way ANOVA with Tukey's multiple comparisons test to compare all groups to one another. Individual values and Mean±SEM are shown. n=6-10 per group

Results

1) Compared to control GF IL-10 KO mice, FMT-inoculated mice demonstrated reduced overall weight gain, elevated levels of fecal lipocalin 2 and calprotectin, and increased colon weight:length ratio at 8 weeks following FMT.
2) Colon protein levels of IL-1β, IL-17, IP-10, MIP-1α, RANTES, and TNFα were elevated in diseased animals.
3) Histopathology and IHC analysis showed that FMT-inoculated mice demonstrated elevated histopathology sum scores, increased colonic CD3+ T cells, CD4+ T_H cells, and increased FoxP3+ $T_{regulatory}$ cells, and epithelial hyperplasia (increased Ki-67 immunolabeling density).
4) Phenotypes were responsive to treatment with anti-p40.

Conclusions

These data provide a validated colitis model with relevant mechanisms for assessing the role of the microbiome and response to therapeutics in IBD.

Studies were performed at BioModels' facility in Waltham, MA, under BioModels' IACUC Protocol 21-1214-1.

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