Histopathological characterization of a chronic DSS-induced mouse model of IBD with intestinal fibrosis

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BACKGROUND & AIM

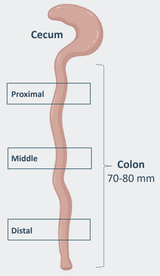
Inflammatory bowel disease (IBD) comprises a group of intestinal disorders, including ulcerative colitis and Crohn's disease. Intestinal fibrosis, as result of chronic inflammation, is a common complication in IBD. High treatment failure rates associated with existing interventions indicate a high unmet need for more effective drugs to improve the management and outcomes of IBD.

Consequently, translational animal models of IBD demonstrating chronic, progressive colonic fibrosis are important tools in preclinical drug discovery for IBD.

The aim of the present study was to characterize intestinal pathology in a chronic DSS-induced mouse model of IBD.

METHODS

10 weeks old male C57BL/6JRj mice were randomized into study groups based on body weight and received 3 cycles of DSS (2.5 % w/v) in the drinking water (DSS-IBD) or normal water (CTRL). Terminal endpoints included colon morphometry, middle colon RNA sequencing/bioinformatic and quantitative histological markers of inflammation and fibrosis assessed in the proximal, middle and distal colonic segment, respectively. In addition, histological analysis was performed for muscularis versus mucosa + submucosa tissue layers in the middle colon.



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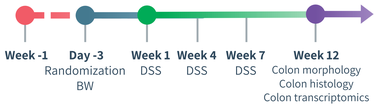
1 Study outline



Group no.	Group	Name	Number of animals
1	Control	CTRL	5
2	DSS	DSS-IBD	14

Figure 1. Study outline.
BW: body weight; DSS: Dextran Sulfate Sodium

2 Body weight and colon morphology

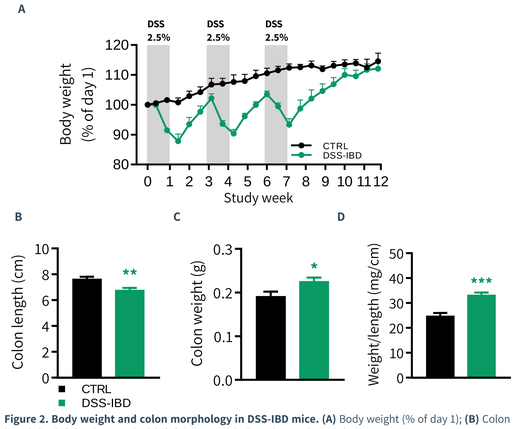


Figure 2. Body weight and colon morphology in DSS-IBD mice. (A) Body weight (% of day 1); **(B)** Colon length (cm); **(C)** Colon weight (g); **(D)** Colon weight/length ratio (mg/cm).

3 Colon inflammation

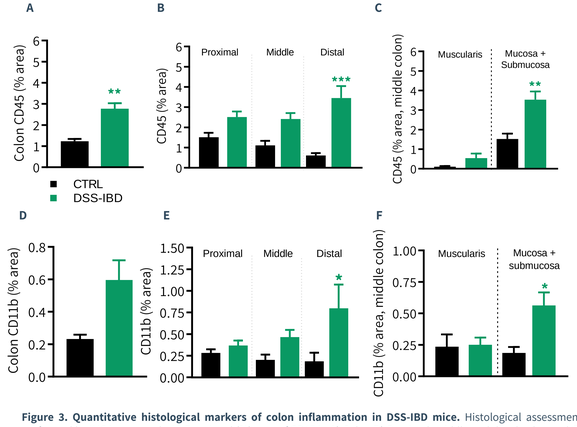


Figure 3. Quantitative histological markers of colon inflammation in DSS-IBD mice. Histological assessment performed by IHC image analysis. Fractional (%) area of CD45 in **A)** Colon **B)** Proximal, middle and distal colon and **C)** middle colon (muscularis, mucosa+submucosa layers).. Fractional area of CD11b in **D**) Colon **E**) Proximal, middle and distal colon and **F)** middle colon (muscularis, mucosa+submucosa layers).

4 Colon fibrosis

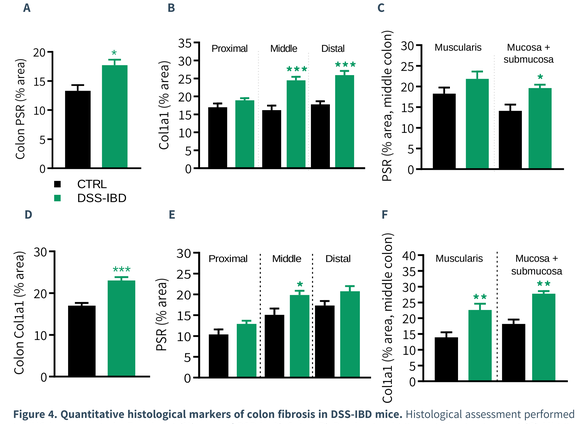


Figure 4. Quantitative histological markers of colon fibrosis in DSS-IBD mice. Histological assessment performed by IHC image analysis. Fractional (%) area of PSR in **A)** Colon **B)** Proximal, middle and distal colon and **(C)** Middle colon (muscularis, mucosa+submucosa layers). Fractional (%) area of Col1a1 in **D)** Colon **E)** Proximal, middle and distal colon and **(F)** Middle colon (muscularis, mucosa+submucosa layers).

5 Colon fibrogenesis

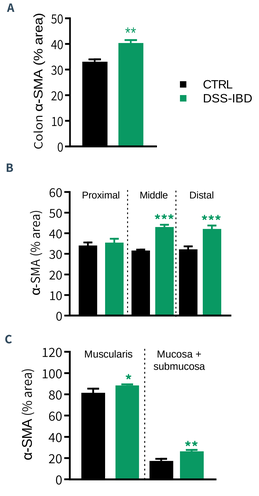


Figure 5. Quantitative histological marker of colon fibrogenesis in DSS-IBD mice. Alpha-smooth muscle actin (α-SMA) fractional (%) area in **A)** Colon **B)** proximal, middle and distal colon; **C)** middle colon (muscularis and mucosa+submucosa layers).

6 Histopathological markers

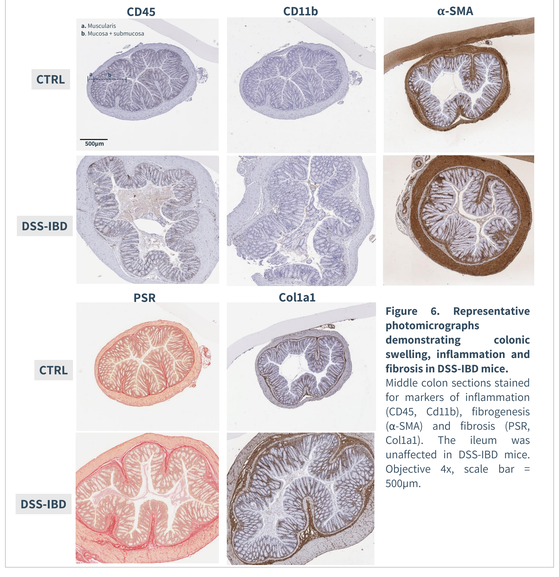


Figure 6. Representative photomicrographs demonstrating colonic swelling, inflammation and fibrosis in DSS-IBD mice. Middle colon sections stained for markers of inflammation (CD45, Cd11b), fibrogenesis (α-SMA) and fibrosis (PSR, Col1a1). The ileum was unaffected in DSS-IBD mice. Objective 4x, scale bar = 500µm.

7 Transcriptome signatures for inflammation and fibrosis

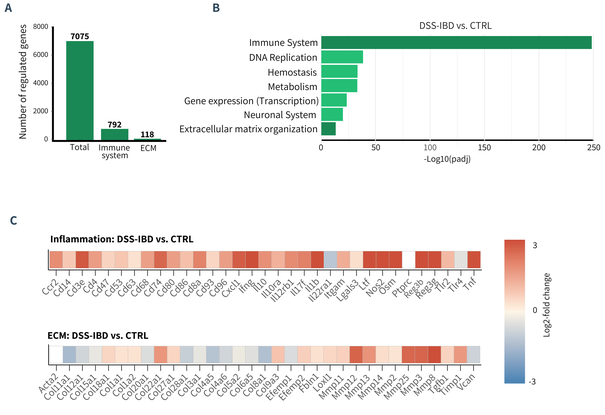


Figure 7. Regulation of inflammation and fibrosis-associated colon gene markers in the DSS-IBD mouse. (A) Total number of differentially expressed genes in DSS-IBD mice compared to CTRL (n=8 per group). **(B)** Overview of top-level Reactome pathways enriched for significantly regulated genes in DSS-IBD mice compared to CTRL ($p<0.05$ after correction for multiple testing). **(C)** Selected inflammation and extracellular matrix (ECM) associated genes (log2-fold change compared to CTRL). Color gradients indicate significantly ($p<0.05$) upregulated (red) and downregulated (blue) genes. White boxes indicate genes not significantly regulated compared to CTRL ($p>0.05$)

CONCLUSION

The chronic DSS-IBD mouse model demonstrates:

+ Mild-to-moderate weight loss and colonic hypertrophy.
+ Marked inflammation in the middle (mucosa, submucosa) and distal (all layers) colon.
+ Sustained, severe fibrosis throughout all layers of the middle and distal colon.
+ Colon transcriptome signatures of inflammation and fibrosis.

The DSS-IBD mouse is a translational preclinical model suitable for testing novel drug therapies for IBD with intestinal fibrosis.