The Impact of Various Gut Microbiota Transfer Methods on the Development of Colitis in Response to Dextran Sulfate Sodium

Keith Lewy, Chunye Zhang, Benjamin Olthoff, Matt Birch, Aaron Ericsson, Craig Franklin
Department of Veterinary Pathobiology
University of Missouri, Columbia, MO 65201, USA

Introduction

- Various methods exist to transfer the gut microbiota (GM) between mice, such as cross fostering and co-housing.
- Currently, the effects of GM transfer method on the final recipient GM composition and overall phenotype are poorly understood.
- As proof of concept, we assessed one such phenotype, the extent of development of colitis upon exposure to dextran sulfate sodium (DSS). The DSS colitis murine model is critical for studying inflammatory bowel disease in humans.

Materials and Methods:

Experimental Groups

SCGM	Microbial Species Richness	Origin/Substrain	Microbial Species Richness
GM1	Low	Jackson Laboratories (B6J)	Low
GM4	High	Envigo (B6NHSD)	High

Table 1. Standardized complex gut microbiota (SCGM) in GM donors (left) and GM species richness of recipient mice (right)

Group	Meaning
B6J GM4CF	B6J mice cross fostered to a CD-1 surrogate dam harboring GM4
B6NHSD GM1CF	B6NHsd mice cross fostered to a CD-1 surrogate dam harboring GM1
B6J GM4CH	B6J mice co-housed at weaning with CD-1 mice harboring GM4
B6NHSD GM1CH	B6Hsd mice co-housed at weaning with CD-1 mice harboring GM1

Table 2. Experimental groups and gut microbiota transfer scheme

Induction of Chronic Colitis

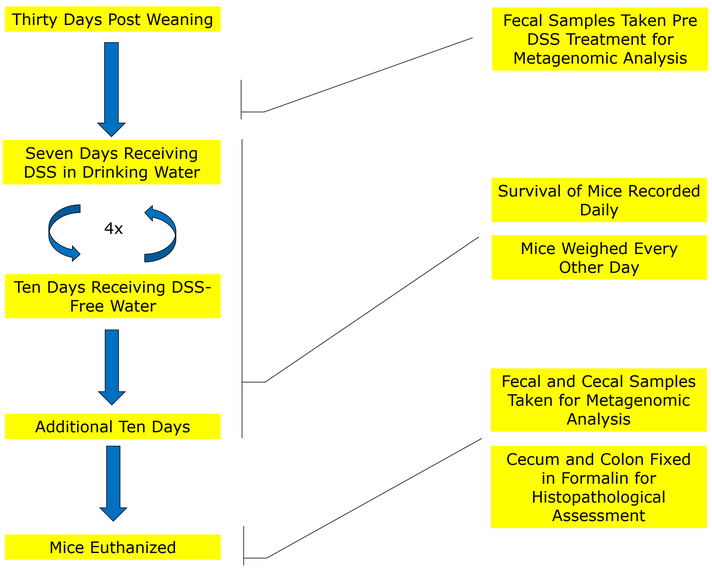

Figure 1. DSS administration to mice

Results:

GM Transfer Method Impact on Final GM Composition

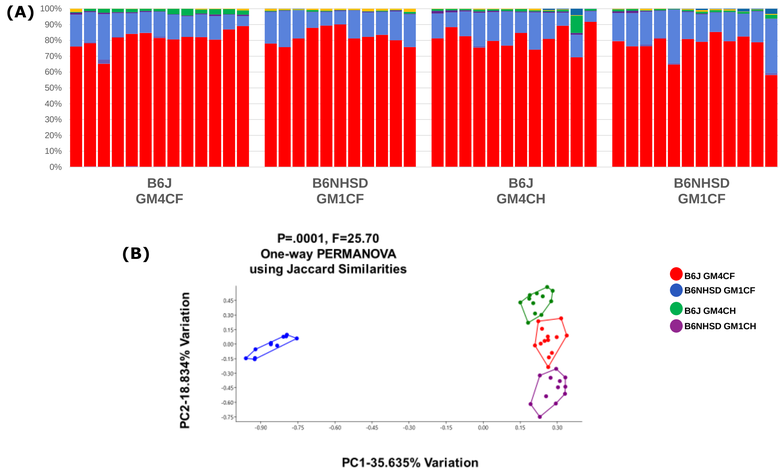

Figure 2. Stacked bar chart showing relative abundance of phyla in feces (**A**) and principal coordinate analysis plots based on Jaccard similarities for fecal microbiota prior to DSS treatment (**B**)

Survival and Weight Loss

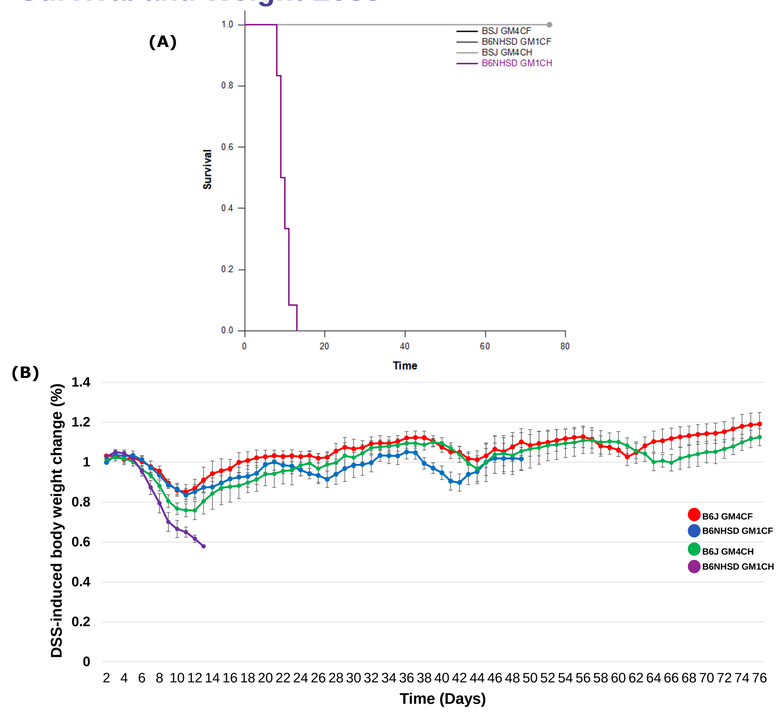

Figure 3. Survival curve (**A**) and weight loss (**B**) for mice used in study. For weight loss analysis significant (P<0.05) differences were found in both main factors (group and time) and interactions between factors (2-way repeated measures ANOVA with Student Newman-Keuls post-hoc analyses

Histological Evaluation of Disease Severity

- The following criteria were used to evaluate severity of colitis:
  - Percent of colon impacted by crypt hyperplasia
  - Percent of colon impacted by crypt dysplasia
  - Foci of mucosal disruption by lymphoid follicles or fibrosis (per 100× field)
  - Lymphoid follicles not disrupting gland architecture (per 100× field)
  - Foci of crypt hyperplasia/dysplasia (per 100× field)
  - Foci of loss of epithelial integrity (erosion or inflamed) (per 100× field)
  - Proximal colon and cecum were analyzed separately from distal colon and rectum

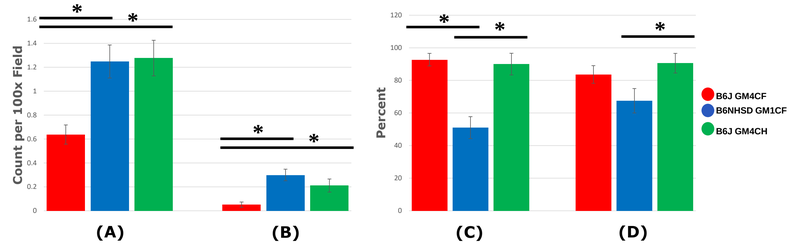

Figure 4. Significant findings: foci of hyperplasia/dysplasia (**A**) and mucosal disruption (**B**) per 100× field in proximal colon/cecum and percent of distal colon/rectum impacted by (**C**) hyperplasia and (**D**) dysplasia

Note: Data were tested for normality using the Shapiro-Wilk test and normally distributed data were analyzed by One Way Analysis of Variance with a Holm-Sidak post hoc test. A p value of 0.05 was considered significant. Non-normally distributed data were analyzed using a Kruskal-Wallis Analysis of Variance on Ranks with Dunn's method for post-hoc analysis.

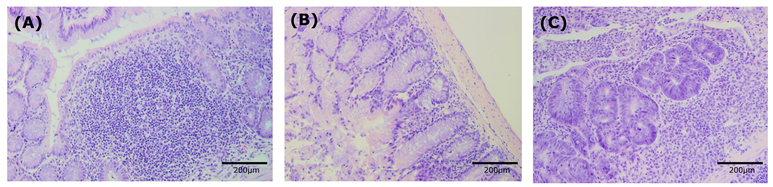

Figure 5. Histological sections of (**A**) mucosal disruption by lymphoid follicle, (**B**) crypt dysplasia (branching), and (**C**) focal crypt hyperplasia/dysplasia at 200×

Conclusions and Discussion

- Different microbiota transfer methods yielded differences in final GM concentrations.
- The early death of all B6NHSD GMCH1 mice and the difference in the rate of weight loss between all groups depending on time point indicates both microbiota transfer method and directionality may impact outward disease severity.
- Differences between all groups were also noted upon histopathological evaluation of different criteria of inflammatory severity. This may shed light onto how both microbiota transfer method and directionality may influence the progression and severity of colitis upon exposure to DSS.
- Current results indicate the need to consider the relative GM richness of donor and recipient mice and GM transfer method in experimental design and analysis and interpretation of 'in house' and published data.

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