Complex Microbial Interactions in DSS-induced Colitis Severity

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Background

Gut microbiota (GM) can change sensitivity and response to disease, immune modulation, and response to treatments. Microbiota varies in populations, communities, and individuals. Different mice from different vendors have a completely different GM makeup

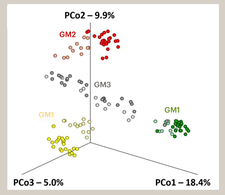


Fig. 1: Three-dimensional principal coordinate analysis plot, based on Jaccard similarities, showing β-diversity captured by the first three coordinates. Mice purchased from Jackson (GM1), Taconic (GM2), Charles River (GM3), and Envigo (GM4).

Microbiota differences are not commonly recorded in the majority of lab animal studies but is important for representative models. With variations between mice of the same strain, there are concerns of reproducibility of studies between labs and institutions. The GM can be manipulated to either increase or decrease transferability of results from mice models.

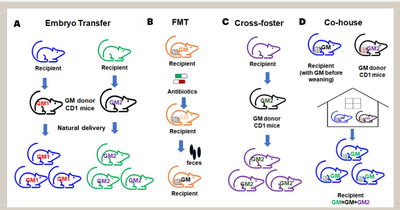


Fig. 2: Different methods of transferring microbiota in laboratory mice.

GM transfer can be done by embryo transfer (ET), fecal microbiota transfer (FMT), cross-fostering (CF), or co-housing (CH). Embryo transfer confers microbiota of dam during and after birth, cross-fostering exposes pups to new dam microbiota less than 24 hours after birth, and co-housing exposes weanlings at 3 weeks old. FMT can be done at any time.

Differences of responses to dextran sulfate-sodium (DSS)-induced colitis have shown to be linked to GM makeup. Previous work has shown that mice transferred by co-housing are more susceptible to DSS than other methods, especially when transferring low richness GM1 to high richness B6N mice.

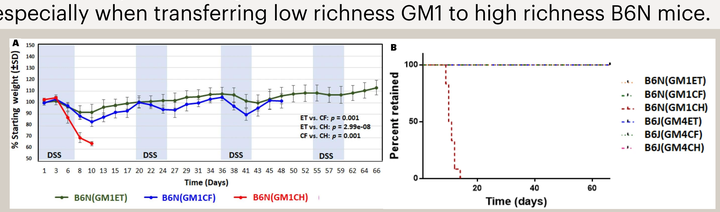


Fig. 3: Influence of transfer method on susceptibility to DSS-induced colitis when transferring low-richness GM1 to B6N mice. **A:** Weight loss. **B:** Percent retained.

Objective and hypothesis

To determine whether differences in disease severity were due to the methods of transfer (co-housing vs gavage + bedding) and/or due to the efficacy of microbiota transfer prior to DSS administration (microbe driven), the following study was designed.

We hypothesized that the difference in disease severity was due to transfer efficiency, and changes in GM would correlate with severity.

Study design and methods

Transfer method was started at weaning. Microbiota transfer took place for 6 weeks, and DSS was administered in drinking water for the last two weeks. At 9 weeks, mice were euthanized, and colons were collected. Feces were collected at 3, 7, and 9 weeks. Cytokine analysis was done on colon samples with ThermoFisher ProcartaPlex Mouse Immune Monitoring Panel 48plex, fecal DNA was extracted using Qiagen QIAamp Power Fecal Pro DNA Kit, and 16S rRNA amplicon libraries were sequenced at MU Metagenomics Center.

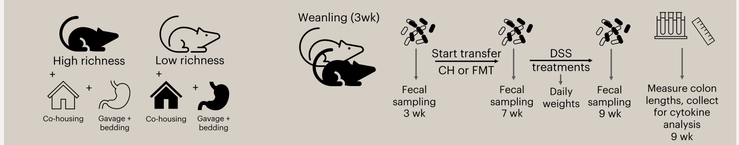


Fig. 4: Weanling C57BL/6 mice with high or low richness GMs were exposed to the opposite GM by co-housing, or by bedding transfer and weekly fecal gavage.

Difference in disease severity between groups

Consistent with previous work, mice receiving low richness GM show significantly greater weight loss and mortality ($p < 0.001$) and shorter colon lengths ($p < 0.001$) than those receiving high richness GM, regardless of transfer method.

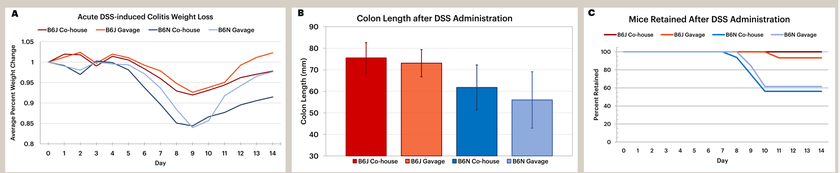
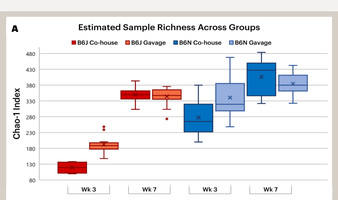


Fig. 5: Disease severity of induced colitis. B6N: high richness recipient mice receiving low richness, B6J: low richness recipient mice receiving high richness. **A:** Average daily weights of mice during DSS administration. Mice with 20% or more weight loss were euthanized. **B:** Colon length after DSS administration. **C:** Percent of mice who did not reach euthanasia endpoint.

Efficiency of different transfer methods

16S rRNA sequencing showed mice GM converged towards the donor GM between 3 and 7 weeks. Jaccard analysis showed significance between strain, recipient and donors, transfer method, and time of collection ($p < 0.0001$), but not sex ($p < 0.1719$)



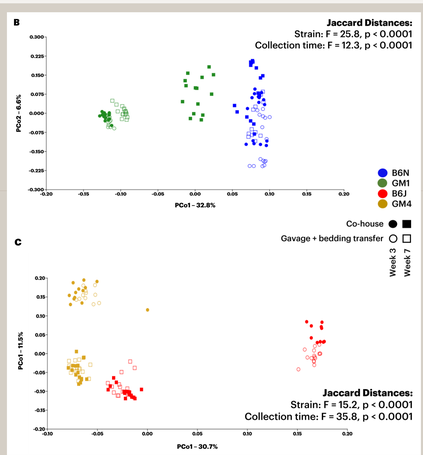


Fig. 6: B6N: high richness recipients, GM1: low Richness donors, B6J: low richness recipients, GM4: high richness donors. **A:** Chao-1 index showing change in estimated richness after GM transfer. **B, C:** Principal coordinate analysis of 16S sequences from fecal DNA of donor vs recipient before and after transfer.

Immune response between groups

Colon samples homogenized and analyzed for cytokine concentration showed an overall difference between the cytokine production levels, with high richness recipient mice receiving a low richness GM showing more pro-inflammatory response then the low mice receiving high richness.

Differences were significant between the high richness recipient and low richness recipient.

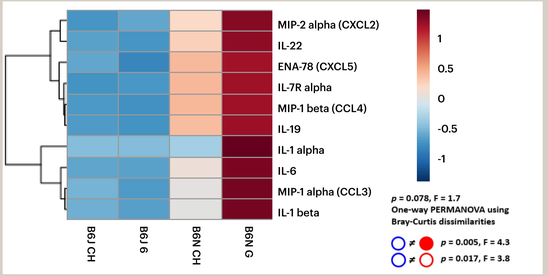
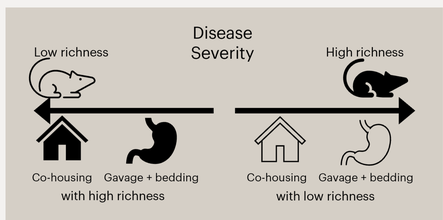


Fig. 7: Cytokine expression levels of colon samples after DSS-induced colitis. B6N: high richness recipient mice receiving low richness, B6J: low richness recipient mice receiving high richness. The top 10 cytokines contributing to the biggest variation between groups shown here.

Conclusions

Directionality of GM transfer impacts the severity of disease, with high richness recipients receiving a low richness GM showing more severe disease. Transferred microbiota colonizes well in recipients with natively low richness, but transfer efficiency is lower in high richness recipients.



We speculate that high richness GM prevents colonization of novel microbes from a low richness donor leading to limited tolerance. Thus, the mucosal immune system is more readily exposed to these immune naive microbes, resulting in a worsening of disease severity.

More research is needed to determine what, if any, group of microbe is driving this immune response, and how the mucosal immune system is responding to the DSS-induced colitis.

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Summary

- **Recipient microbiota has significant impact on DSS-induced colitis severity**
- **Transfer of microbiota using co-housing or gavage, and direction of transfer influences severity**
- **Classifying microbiota, like classifying mouse strain is essential for reproducibility, transferability, and animal health**