# Maternal and Microbial Flora Factors in Inflammatory Bowel Disease

K. Jamie Holland, Andrew Hillhouse, Lisa Zell, Craig Franklin, DVM, PhD

## Abstract

It is estimated that one million Americans suffer from inflammatory bowel diseases (IBDs). These diseases are idiopathic; however, genetic, environmental, and microbial flora factors are suspected to contribute. Mice infected with *Helicobacter hepaticus* develop chronic inflammation of the large intestine and thus represent an attractive animal model of IBD. Importantly, the A/J strain of mice is susceptible to disease, whereas the C57BL/6 (B6) strain is resistant; this allows for the study of both genetic and environmental factors in disease susceptibility. Using this model, segments of specific chromosomes have been identified that contribute to, but do not entirely account for, disease susceptibility. Consequently, we are examining whether other factors (microbial and maternal) may also contribute to disease susceptibility. To assess microbial factors, cecal contents from A/J and B6 mice were cultured and assessed for qualitative and quantitative differences. To evaluate whether host intestinal products (e.g. defensins) shape microbial flora, cecal contents from both strains will be cultured in the presence of intestinal homogenates from both strains. To assess whether maternal factors affect disease, A/J mice are being cross-fostered to B6 mice and vice versa. Disease susceptibility is being evaluated by examining cytokine production and chronic lesion development. We anticipate that microbial flora from A/J and B6 mice will differ, qualitatively and quantitatively, and that differences in intestinal epithelial products between these strains may shape this flora. We also anticipate that cross fostering of mice will modify disease susceptibility, and speculate that this maternal influence may be related to microbial flora alterations.

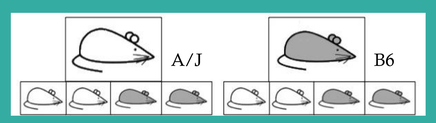
## Methods

Microbial Flora Experiment

Cecal contents from young mice (3-4 weeks) and old mice (6-7 months) were isolated and 0.06 g were diluted in 1ml of PBS. The cecal solution was homogenized and centrifuged at 200xG for 1 minute. One hundred microliters of a 1:1000 dilution was pipetted onto blood agar plates and spread evenly. The plates were incubated for 2 days at 37°C in both aerobic and anaerobic conditions. Isolates were speciated using standard microbiological and biochemical methodologies.

Cross-foster Experiment

A/J and B6 mice were placed in breeding trios (2 females and 1 male). Litters born within one day of each other were cross-fostered; half of B6 pups were left with original mother (as controls) and the other half were cross-fostered with an A/J mother. This procedure was repeated for the A/J litters.

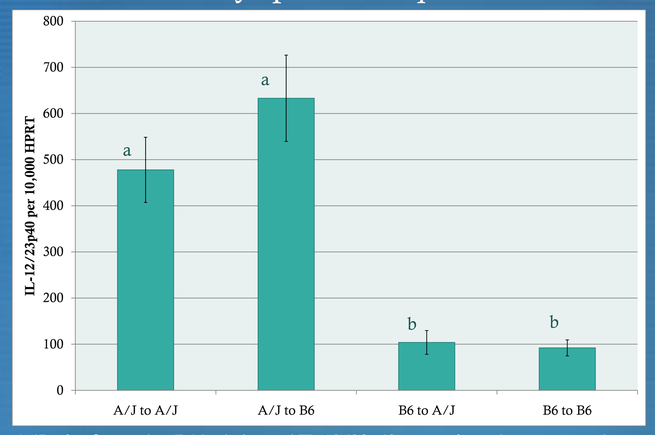



Pups were weaned at 3 weeks and inoculated with approximately $10^8$ *Helicobacter hepaticus* via oral gavage. Maternal effects on host response to *H. hepaticus* were assessed by measuring cecal IL-12/23p40 gene expression at 4 days post-inoculation (PI) and cecal IL-12/23p40 expression and lesion development at 90 days PI. For cytokine expression analysis, mRNA was prepared from cecal tissues, cDNA was generated and levels of cecal IL-12/23p40 expression estimated by quantitative real-time PCR (normalized to the expression of the house keeping gene HPRT). Lesion development was assessed histologically using a lesion scoring system previously described (Myles et al. Infection and Immunity, 2003. Vol 71 No. 7. p. 3885-3893).

## Results

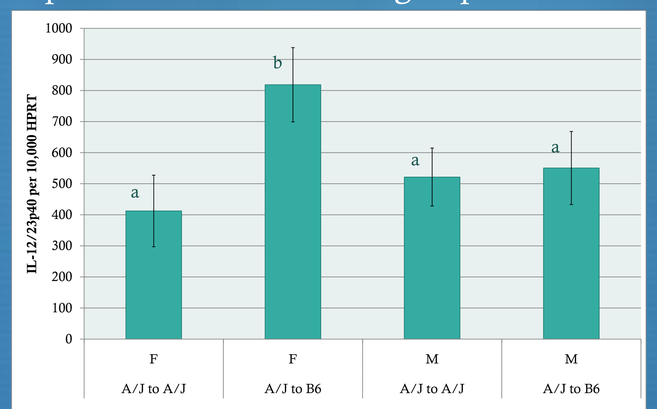
### Assessment of Host Immune Response

Figure 1. Cecal IL-12/23p40 expression in cross-fostered mice 4 days post- *H. hepaticus* inoculation.




A/J mice fostered to B6 had elevated IL-12/23p40 expression when compared to A/J mice fostered to A/J mice; however this difference was not statistically significant. Means with different letters are significantly different (1-way ANOVA, $p < 0.05$).

Figure 2. Sex factors in cecal IL-12/23p40 expression in fostered A/J groups.




Since we saw a trend towards increased cecal IL-12/23p40 expression in A/J mice fostered to B6 mice, we assessed whether sex influenced this data. This analysis revealed that female A/J mice fostered to B6 mothers had significant elevations in IL-12/23p40 expression when compared to female A/J mice fostered to A/J mothers ($p<0.05$). No such difference was seen in male mice. Means with different letters are significantly different (2-way ANOVA, $p<0.05$).

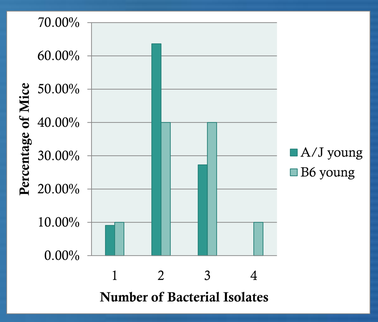
### Assessment of Flora Differences

Table1. Culturable bacterial isolates from young and old A/J and B6 mice.

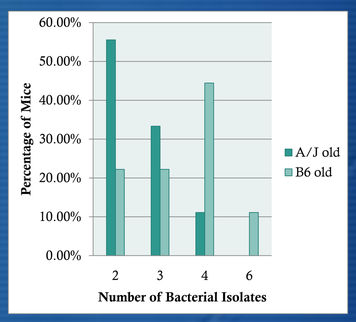
| Bacterial Isolate | Young A/J | Young B6 | Old A/J | Old B6 |
|---|---|---|---|---|
| *Lactobacillus* sp. | 11/11 (100%) | 10/10 (100%) | 6/9 (67%) | 7/9 (78%) |
| *Enterococcus faecalis* | 1/11 (9%) | 8/10 (80%) | 5/9 (56%) | 7/9 (78%) |
| *Staphylococcus xylosus* | 3/11 (27%) | 3/10 (30%) | 4/9 (44%) | 4/9 (44%) |
| Alpha hemolytic *Streptococcus* sp. | 9/11 (82%) | 4/10 (40%) | 7/9 (78%) | 7/9 (78%) |
| *Escherichia coli* | 0/11 (0%) | 0/10 (0%) | 0/9 (0%) | 6/9 (67%) |
| *Bacteriodes thetaiotamicron* (anaerobic) | 11/11 (100%) | 10/10 (100%) | 7/9 (78%) | 7/9 (78%) |

Eighty perecent of young B6 mice were positive for *Enterococcus faecalis* whereas only 9% of A/J mice were positive for this bacterium. Eighty two percent of young A/J mice were positive for an alpha hemolytic *Streptococcus* species while only 40% of B6 mice were positive for this bacterium. In old mice, the majority of the B6 mice (67%) were positive for *E. coli*, whereas no A/J mice were positive for this bacterium. There was also a slight increase in the incidence of *Enterococcus faecalis* in old B6 mice when compared to old A/J mice.

Figure 3a and b. Comparison of the number of different bacterial isolates cultured from young and old A/J and B6 mice .




The number of bacterial isolates was similar between young A/J and B6 mice, however there were more B6 mice with 3 or more bacterial isolates than A/J mice.




Only two bacterial isolates were found in the majority of old A/J mice, whereas 4 bacterial isolates were found in the majority of B6 mice.

## Conclusions

- There is a significant maternal effect on A/J females mice fostered onto a B6 mothers when compared to A/J females fostered to A/J mothers.
- This maternal effect of B6 mice may represent a complex interaction between the sex of the pup and maternal factors such as milk, mothering style, or bacterial flora.

## Future Directions

- Examine cecal flora in cross-fostered mice
- Ninety day post-inoculation cross-fostered mice
  - Examine cecal IL-12/23p40 levels
  - Histologically evaluate cecal lesion scores

## Conclusions

- The incidence of *Enterococcus faecalis* was higher in young B6 mice when compared to young A/J mice.
- The majority of old B6 mice were positive for *E.coli*, however no old A/J mice were positive.
- The incidence of alpha hemolytic *Streptococcus* sp. was higher in young A/J mice when compared to young B6 mice.
- B6 mice tended to have more isolates per mouse than A/J mice, suggestive of a more complex flora.

## Future Directions

- Culture additional A/J and B6 mice to confirm trends
- Investigate antibacterial effects of epithelial products
  - Examine the effect of ileal homogenates from both strains on growth of various isolates

## Acknowledgements

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