

Original Aims:

Specific Aim 1 - Identify microbes from mice that induce UC in healthy *dnKO* mice. We will utilize 'healthy' *dnKO* mice (treated with antibiotics to eliminate disease) as recipients for donor intestinal microbes from donor mice. We will manipulate and refine the donor population of microbes that can elicit disease by a sequence of approaches. After this iterative refinement of the colitogenic microbial population, we will elucidate the species that are present and begin to define their interaction with the mucosal immune system.

Specific Aim 2 – Identify microbes from acute pouchitis patients that induce UC in healthy *dnKO* mice. Patients with acute pouchitis, a form of IBD, typically respond to Mtz/Cf treatment as do the *dnKO* mice. Because of this similarity, we will test if antibiotic-sensitive colonic microbes from patients with acute pouchitis (and healthy controls) can stimulate colitis in the *dnKO* mice. If this occurs, we will attempt to identify the colitogenic microbe(s).

We focused our efforts exclusively on Aim 1, due to the success of developing a system to screen for microbes that induced colitis in this system. Aim 2 is a project that we will pursue in the future.

Accomplishments/Significant Findings:

- Non-germ-free screen for disease-inducing bacteria in a genetic mouse model of IBD
- Common commensal *Bacteroides* fulfill host-genotype-specific Koch's postulates
- Commensal Enterobacteriaceae are enriched during spontaneous disease
- Enterobacteriaceae not sufficient to induce disease despite robust colonization

Lay Summary:

Inflammatory bowel disease (IBD) arises from complex interactions of genetic, environmental, and microbial factors. The intestinal microbiota is known to be crucial for disease induction. However despite a wealth of data on IBD-associated shifts in microbiota composition, it remains unclear to what extent, if any, specific intestinal microbes induce disease. To address this question we established a screen using a highly penetrant, rapid-onset mouse model of IBD with genetic features relevant to human disease. Antibiotics blocked disease induction in this model and mice remained disease-free after halting antibiotic treatment. To fulfill Koch's postulates we isolated intestinal bacteria, experimentally introduced them into antibiotic-pre-treated mice, and confirmed colonization by quantitative re-isolation in culture. In this study, we showed that common commensal *Bacteroides* species induced disease exclusively in genetically susceptible hosts whereas an Enterobacteriaceae species profoundly enriched in spontaneously colitic mice was not itself sufficient for disease induction. Antibiotic-pre-treated susceptible mice developed colitis if colonized with intestinal contents from antibiotic-naïve animals, mixed bacterial cultures of intestinal contents, or commensal intestinal *Bacteroides* isolates. Antibiotic-pre-treated non-susceptible animals given the same bacterial isolates remained colitis-free despite becoming stably colonized at levels equivalent to their susceptible littermates. In contrast, although we observed dramatic enrichment of commensal Enterobacteriaceae in antibiotic-naïve (spontaneously colitic) susceptible mice, an Enterobacteriaceae isolate was not sufficient for disease induction in antibiotic-pre-treated animals despite robust colonization. These findings demonstrate IBD induction by a specific subset of commensal intestinal bacteria, emphasize that IBD-associated microbiota alterations are not necessarily predictive of disease etiology, and establish both experimental criteria and a conceptual framework for identifying components of the commensal microbiota with disease-inducing potential.