

It is becoming increasingly apparent that the microbiota play a critical role in inflammatory bowel diseases such as Crohn's and colitis. A recent study of human microbial communities showed that there is a significant drop in numbers of intestinal microbiota as well as a shift in constituents in patients with IBD (PNAS, 2007, 104:13780-85). However, the interplay between intestinal inflammation and the microbiota has not been extensively studied in IBD, and specific microbiota constituents associated with susceptibility or resistance to disease have not been established.

We have found that there are very similar decreases in microbiota numbers and similar shifts in flora population in an experimental mouse colitis model using *C. rodentium* (Appx. 1) compared to the human results above. The microbiota composition, at a phylum level, is surprisingly similar between mice and humans. This murine model resembles IBD in several ways, including a polarized Th1 response, similar cytokine responses, subsequent intestinal inflammation, and importantly similar shifts in microbiota. We also found that if we altered inflammation by using either chemical modulators (DSS) or altered host immune inflammatory responses (hypo and hyper inflammatory), the susceptibility to colitis was significantly altered.

Recently we have developed an excellent murine model of chronic colitis and intestinal fibrosis, using a longer term infection with *S. Typhimurium*. Fibrosis is a major complication in IBD, and this model mimics human fibrosis in many respects, including collagen deposition and a Th1/Th17 cytokine response. A major advantage of this model is that the fibrosis resolves. Thus, by monitoring shifts in microbiota during infection and resolution, one can better determine the shifts in microbiota associated with disease.

By using these experimental models of colitis in defined murine backgrounds, we hope to determine a much better understanding of microbiota constituents associated with susceptibility and resistance to disease. The advantage of this experimental system is that each of the components can be manipulated experimentally. The microbiota can be shifted by various antibiotics, and we have characterized these shifts and now will determine their effects on colitis. The pathogen can be altered, and we have done much work on altering various virulence factors in both these pathogens, and have even generated a graded set mutants with decreasing virulence capacity, thus providing a range of disease-causing organisms. The host's inflammatory response can be altered by using genetically altered mice containing mutations in key inflammatory pathways.

We feel we are now in an excellent position to further define the role of inflammation on microbiota composition in the context of experimental colitis. Because each of the three components (microbiota, pathogen, inflammation) can be experimentally altered, one can probe various aspects of the interplay between inflammation and microbiota. By the completion of this grant, a list of constituent microbiota associated with increased or decreased colitis will be defined. This list can be compared to results with human microbiota in normal and IBD individuals. Ultimately and ideally, one or more microbiota constituents may be identified that are specifically associated with IBD or resistance to disease.

Although both inflammation and microbiota are known to be contributing factors to IBD, the interplay between the two during IBD is not well understood. For example, whether inflammation triggers the shift in microbiota, or shifts in microbiota affect the subsequent inflammation is not defined. By employing our experimental models, these questions can be specifically addressed. Moreover, by using reversible disease models, one can study both the onset and resolution of disease, providing a more comprehensive model to understand the interplay between inflammation and microbiota and their effects on acute and chronic colitis as well as fibrosis.

Collectively, this work will provide much information on the role of inflammation and the microbiota on colitis. Because we are using relevant animal models, the majority of concepts and information generated here should be directly applicable to human IBDs.