molecules generated from microbial metabolism, knowledge of how inflammation alters the microbial metabolism and how epithelial cells react is important for a better understanding of how IBD develops and persists. Butyrate, a short chain fatty acid produced through fermentation of dietary polysaccharides, has long been known to inhibit histone deacetylases (HDACs), which represent one of the many types of enzymes responsible for the epigenetic control of gene expression through the post-translational modification of histone proteins. We and others have observed that colonic epithelial cells from germ-free mice have reduced levels of acetylation on histone H4, which appears to be distributed throughout the genome based on sequencing analysis. The decreased levels of H4 acetylation may stem from a lack of butyrate, and therefore inhibited HDAC activity, in germ-free mice. However, since colonic epithelial cells utilize short chain fatty acids as an energy source, an alternative explanation is that the germ-free condition results in less oxidation of butyrate to acetyl-CoA, which is the donor substrate for histone acetylation reactions. Isotope tracing experiments, in which cultured cells were incubated with labeled butyrate, demonstrated that the acetyl groups of histones contained carbon derived from butyrate. We have also performed isotope tracing experiments in mice using labeled inulin, a plant polysaccharide that presumably undergoes fermentation in short chain fatty acids. In this more physiologically relevant model, we detected isotope incorporation into the acetylation products of histone H4 at rates of 5–20%, which appears dependent on the microbiota since labeling is sensitive to antibiotic treatment. To identify the metabolic pathways that link inulin to histone acetylation, we are investigating which metabolites become isotopically labeled using untargeted metabolomics. We will apply the same approach to the DSS-induced model of colitis to investigate how inflammation modulates the gut microbiome as well as the metabolic connections between the microbiota and the host. Our studies may uncover metabolic pathways that become dysregulated during inflammation, which may contribute to the pathogenesis of diseases such as IBD.

7 LACTOBACILLUS REUTERI SUPPRESSES PRO-INFLAMMATORY DRIVEN REACTIVE OXYGEN SPECIES IN VITRO IN HUMAN INTESTINAL EPITHELIAL CELLS AND IN VIVO IN A TNBS COLITIS MOUSE MODEL

Wenly Ruan, Melinda Engevik, Alexander Chang-Graham, Joseph Hyser, James Versalovic

Background: Reactive oxygen species (ROS) play a role in maintaining intestinal epithelial homeostasis and are normally kept at low levels via antioxidant compounds. Dysregulation of ROS can lead to intestinal inflammation and contribute to inflammatory bowel disease (IBD). Select gut microbes possess the enzymatic machinery to produce antioxidants whereas others can dysregulate levels of ROS. Our model microbe, Lactobacillus reuteri (ATCC PTA 6476), has been demonstrated to reduce intestinal inflammation in mice models. It contains the genes encoding two distinct GshA-like glutamylcysteine ligases. We hypothesize that L. reuteri can secrete y-glutamylcysteine to suppress ROS, minimize NFkB activation and regulat e pro-inflammatory cytokine driven ROS and IL-8 production. To further test the role of L. reuteri in vivo we generated γ-glutamylcysteine deficient mutants by targeted mutagenesis of the γ-glutamylcysteine. HT29 cells and HJE were then treated with IL-1β or hydrogen peroxide, and isolation of γ-glutamylcysteine as an energy source, an alternative explanation is that the germ-free condition results in less oxidation of butyrate to acetyl-CoA, which is the donor substrate for histone acetylation reactions. Isotope tracing experiments, in which cultured cells were incubated with labeled butyrate, demonstrated that the acetyl groups of histones contained carbon derived from butyrate. We have also performed isotope tracing experiments in mice using labeled inulin, a plant polysaccharide that presumably undergoes fermentation in short chain fatty acids. In this more physiologically relevant model, we detected isotope incorporation into the acetylation products of histone H4 at rates of 5–20%, which appears dependent on the microbiota since labeling is sensitive to antibiotic treatment. To identify the metabolic pathways that link inulin to histone acetylation, we are investigating which metabolites become isotopically labeled using untargeted metabolomics. We will apply the same approach to the DSS-induced model of colitis to investigate how inflammation modulates the gut microbiome as well as the metabolic connections between the microbiota and the host. Our studies may uncover metabolic pathways that become dysregulated during inflammation, which may contribute to the pathogenesis of diseases such as IBD.

Methods & Results: Conditioned media from L. reuteri was analyzed via mass spectrometry to confirm the presence of y-glutamylcysteine. All cysteine containing products including γ-glutamylcysteine were fluorescently tagged in the conditioned media and then incubated with HT29 cell monolayers as well as human jejunal enteroid (HJE) monolayers. γ-glutamylcysteine was demonstrated to enter the intestinal epithelial cells based on microscopy. Next, a Thioltracker assay was used to show increased intracellular glutathione levels by L. reuteri secreted γ-glutamylcysteine. HT29 cells and HEs were then treated with IL-1β or hydrogen peroxide, and L. reuteri metabolites as well as γ-glutamylcysteine significantly suppressed pro-inflammatory cytokine driven ROS and IL-8 production. L. reuteri secreted products also reduced activity of NFkB as determined by a luciferase reporter assay. γ-glutamylcysteine deficient mutants were generated by targeted mutagenesis of GSHA genes, and these mutant L. reuteri strains had a diminished ability to suppress IL-8 production and ROS. To further test the role of L. reuteri secreted γ-glutamylcysteine in vivo, a 2,4,6-Trinitrobenzenesulfonic acid (TNBS)- induced mouse colitis model was used. Adolescent mice were orogavaged with PBS, L. reuteri, L. reuteri GshA2 mutant, or γ-glutamylcysteine for a week after which TNBS was rectally administered to induce colitis. We demonstrate that L. reuteri and γ-glutamylcysteine can suppress histologic inflammation compared to PBS control and L. reuteri GshA2 mutant groups.

Conclusions: Together these data indicate that L. reuteri secretes γ-glutamylcysteine which can enter the intestinal epithelial cells and modulate epithelial cytokine production. It acts via suppression of ROS and NFkB which then decreases IL-8 production. We are able to demonstrate this in vivo in both HT-29 cells and HEs. We now also demonstrate this in vivo in a mouse colitis model. These experiments highlight a prominent role for ROS intermediates in microbiome-mammalian cell signaling processes involved in immune responses and intestinal inflammation.

3 MECHANISMS OF INTESTINAL FUNGI RECOGNITION AND CONTROL

Irina Leonardi, Illya Ilyev

Background and Objectives: Intestinal fungal communities are perturbed in several autoimmune diseases and have been shown to influence disease outcome. We have shown that intestinal resident CX3CR1+ mononuclear phagocytes (MNP)s can sense gut fungi and are crucial for the initiation of immune responses both locally and at distant sites. These results suggest that recognition of fungi by gut phagocytes might be involved in the pathogenesis of immune-related diseases such as IBD. Despite the identification of a few molecules involved in the recognition and immunity to intestinal fungi the cellular mechanisms governing the initiation and regulation of the mucosal immune responses to the mycobiota remain unknown. We sought to identify the functional role of distinct phagocytic subsets in the response to fungal communities in the gut during health and intestinal disease.

Methods: We used Candida albicans, the main opportunistic fungus found in IBD patients, as model fungal colonizer, with a focus on the mechanisms mediating the adaptive response. To elucidate the mechanisms and consequences of the recognition of fungi by phagocytic subsets in the lamina propria we used genetic models of deletion and deletion of specific subsets of phagocytes. We further targeted fungal recognition and antigen presentation in phagocytes to investigate the adaptive immune response leading to the induction of adaptive T cell responses. Results: C. albicans colonization induced a consistent increase in Th17 cells in the intestinal mucosa that was decreased upon deletion of CX3CR1+ MNP-s. Genetic deletion of CX3CR1+ MNP-s in mice led to changes in gut fungal communities and increased chemically induced intestinal inflammation that was rescued by anti-CD11b treatment. Recognition of fungi through a C-type lectin/Syk pathways and antigen presentation via MHC-II were necessary for the induction of adaptive T cell in responses to C. albicans colonization.

Conclusions: Our work aims at defining the role of CX3CR1+ MNP-s and cDCs in the initiation of anti-fungal immune responses in the intestine at the steady state. We have demonstrated the essential role of CX3CR1+ MNP-s in the initiation of antifungal responses in the intestine at steady state and for the control of fungi at steady state and during inflammation. We have demonstrated the importance of CX3CR1+ MNP-s in the control of the intestinal mycobiota.
Discussion: Patients with UC and response to therapy had a significantly different pre-treatment microbiome and methylation of genes related to intestinal barrier function, including B4GALT1. Larger studies will be needed to validate these findings, but these results suggest the microbiome and DNA methylation changes may be effective biomarkers of response to therapy and warrant further study.

8
ROLE OF THE HOST IMMUNE RESPONSE TO ENTERIC PROKARYOTIC VIRUSES IN INFLAMMATORY BOWEL DISEASE

Julia Angkew, Daniel Monaco, Scott Handley, H.B. Larman

Background: Gut microbiota comprise important environmental exposures that influence human immune systems and may alter the clinical course of inflammatory bowel disease (IBD). Little is known about the role of gut bacteriophages (viral components that infect prokaryotic bacteria) and their interactions with the host’s immune responses. We tested the hypotheses that (1) immune responses of individuals with IBD to phages differ from those without IBD and (2) immune responses to phages are associated with disease type (i.e. those with Crohn’s disease have different responses than those with ulcerative colitis).

Methods: We have constructed the first bacteriophage peptide library (“phagome”), based on sequencing of environmental phages and large-scale metagenomic sequencing of virus-like particles isolated from stool samples from IBD patients and their non-IBD household contacts. Using Phage ImmunoPrecipitation Sequencing (PhIP-Seq) technology, we generated complete serum antibody binding profiles of 48 IBD patients (16 ulcerative colitis, 11 Crohn’s, and 11 indeterminate), 9 of their non-IBD household contacts, and an independent non-IBD cohort of 674 volunteers collected by the Vaccine Research Center (VRC) at the National Institutes of Health. Antibody binding profiles were compared among groups using nonparametric statistics.

Results: IBD patients as a group had lower antibody responses to specific phages compared to both non-IBD household contacts and the non-IBD VRC controls; this difference was significant and remained after control for unequal sample sizes (Figure 2). IBD disease type comparisons to the VRC controls yielded similar results. Conclusion: PhIP-Seq with a phageome library can be used to study the relationship between immune responses and gut bacteriophages in IBD. Our results suggest that IBD patients may have lower antibody responses to specific phages compared to non-IBD individuals. Differential antibody reactivities in Crohn’s disease vs. ulcerative colitis compared to their household contacts and VRC controls suggest disease-specific response to the gut phageome that warrant further study.

10 THE ROLE OF DIETARY L-SERINE IN THE REGULATION OF INTESTINAL MUCUS BARRIER DURING INFLAMMATION

Kohel Sugihara, Nobuhiko Kamada

Background: Recent accumulating evidence suggests that amino acids have crucial roles in the maintenance of intestinal homeostasis. In inflammatory bowel disease (IBD), amino acid metabolism is changed in both host and the gut microbiota. Among amino acids, L-serine plays a central role in several metabolic processes that are essential for the growth and survival of both mammalian and bacterial cells. However, the role of L-serine in intestinal homeostasis and IBD remains incompletely understood. In this study, we investigated the effect of dietary L-serine on intestinal inflammation in a murine model of colitis.

Methods: Specific pathogen-free (SPF) mice were fed either a control diet (amino acid-based diet) or an L-serine-deficient diet (SDD). Colitis was induced by the treatment of dextran sodium sulfate (DSS). The gut microbiome was analyzed by 16S rRNA sequencing. We also evaluated the effect of dietary L-serine in germ-free mice and gnotobiotic mice that were colonized by a consortium of non-mucolytic bacterial strains or the consortium plus mucolytic bacterial strains.

Results: We found that the SDD exacerbated experimental colitis in SPF mice. However, the severity of colitis in SDD-fed mice was comparable to control diet-fed mice in germ-free condition, suggesting that the gut microbiota is required for exacerbation of colitis caused by the restriction of dietary L-serine. The gut microbiome analysis revealed that dietary L-serine restriction fosters the blooms of a mucus-degrading bacterium Akkermansia muciniphila and adherent-invasive Escherichia coli in the inflamed gut. Consistent with the expansion of mucolytic bacteria, SDD-fed mice showed a loss of the intestinal mucus layer. Dysfunction of the mucus barrier resulted in increased intestinal permeability, thereby leading to bacterial translocation to the intestinal mucosa, which subsequently increased the severity of colitis. The increased intestinal permeability and subsequent bacterial translocation were observed in SDD-fed gnotobiotic mice that colonized by mucolytic bacteria. In contrast, dietary L-serine restriction did not alter intestinal barrier integrity in gnotobiotic mice that colonized only by non-mucolytic bacteria.

Conclusion: Our results suggest that dietary L-serine regulates the integrity of the intestinal mucosal barrier during inflammation by limiting the expansion of mucolytic bacteria.