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 diet, possesses anti-inflammatory properties.

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

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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 6475), 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 γ-glutamylcysteine to suppress ROS, minimize NFkB activation and regulate intestinal cytokines.

Methods & Results: Conditioned media from L. reuteri was analyzed via mass spectrometry to confirm the presence of γ-glutamylcysteine. All cytokine containing products including γ-glutamylcysteine were fluorescently tagged in the conditioned media and then incubated with HT29 cell monolayers as well as human intestinal epithelial cells. 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 NFκB 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 orogastrically fed PBS, L. reuteri, L. reuteri GhSA2 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 GhSA2 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 NFκB which then decreases IL-8 production. We are able to demonstrate this in vivo in both HT29 cells and HEp-2 cells. 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

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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 (MNPs) 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 mycobacteria 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 subtypes of phagocytes. We further targeted fungal recognition and antigen presentation in phagocytes to investigate the role of phagocytic subsets in the induction of adaptive responses leading to the induction of anti-fungal immune responses.

Results: C. albicans colonization induced a consistent increase in Th17 cells in the intestinal mucosa that was decreased upon deletion of CX3CR1+ MNPs. Genetic depletion of CX3CR1+ MNPs in mice led to changes in gut fungal communities and for the first time chemically induced intestinal inflammation that was rescued by antifungal 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 responses to C. albicans colonization.

Conclusions: Our work aims at defining the role of CX3CR1+ MNPs 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+ MNPs 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+ MNPs in the control of the intestinal mycobacteria.

P166 MICROBIOME AND EPIGENETIC PREDICTORS OF RESPONSE TO BIOLOGIC THERAPY IN PATIENTS WITH ULCERATIVE COLITIS

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Introduction: Current ulcerative colitis (UC) treatments have variable efficacy and may take several weeks to assess improvement. Emerging data suggest the intestinal microbiota may serve as a biomarker and mechanistically may influence immune system activity through epigenetic regulation of host gene expression. The aim of this pilot project was to examine whether the colonic mucosal microbiota and mucosal DNA methylation patterns are associated with a response to treatment in UC.

Methods: We conducted a retrospective cross-sectional study of patients with active UC. Fresh frozen colon biopsy samples were obtained from the Texas Medical Center IBD Tissue Bank. Patients were included if they had a colonoscopy performed to assess disease activity and follow up through 14 weeks to assess response. Disease activity was defined using the Partial Mayo Score and response was defined as a 2 or greater decrease in the score after 14 weeks of follow up. 16s rRNA gene sequencing and DNA methylation pyrosequencing were used to define the microbiome and quantify DNA methylation. Comparisons were performed of alpha and beta diversity, taxonomy and DNA methylation between responders and non-responders at 14 weeks. Additionally, a random forest machine learning model was developed to identify predictors of response.

Results: We identified 16 patients with tissue samples from the cecum/ascending, transverse and rectum/sigmoid available for analysis. After excluding patients with limited rectal disease, recent antibiotic use and inadequate follow up, 4 patients (2 per group) were included. We studied total 12 tissue samples for analysis. The mean age of responders was 38 and non-responders was 24. 50% of patients were male and all were Caucasian. Responders had a numerically lower alpha diversity, though this did not reach statistical significance (p=0.18). Responders and non-responders separated in unweighted (p=0.001) and weighted (p=0.001) beta diversity analysis. Responders had a greater abundance of Firmicutes and Bacteroidetes and lower abundance of Proteobacteria compared to non-responders. This corresponded to responders having a greater abundance of genera for Bifidobacterium, Fecalibacterium and Roseburia. Additionally, we saw increased methylation in the P16, HOX5 and B4GALNT1 genes of responders compared to non-responders. Finally, in a random forest machine learning model, predictors of response included left sided disease extent and OTUs for the genus Roseburia.