A NOVEL ROLE OF THE STOMACH IN PROTECTION FROM COLITIS
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Background: Gastrin (Gln3) is an 18 kDa protein produced and secreted into the lumen in the antrum of the stomach. It is a stable, protease-resistant protein, suggesting that it can resist degradation in the stomach and throughout the GI tract. Exogenous Gln1 peptide has been shown to be beneficial to the health of the distal gut. We examined the specificity of Gln1 production in the stomach and its impact on the distal gut microbiome. Recombinant Gkn1 peptide also protects mice from colitis. We tested the requirement for Gln1 in the protection from T-cell mediated intestinal inflammation in a mouse model of colitis. Methods: Gln1 protein and mRNA levels were assessed in an array of tissue types from mice. 8 week old WT and Gln1-/- mice were sensitized to 2,4,6-trinitrobenzenesulfonic acid (TNBS) 1 week prior to rectal infusion of a 2.5% (w/v) solution of TNBS and ethanol to induce T-cell mediated colitis, or control solution of ethanol alone. Mice were necropsied at either 2 or 7 days post TNBS administration and assessed for the development of colitis. Colonic contents were collected from untreated WT and Gln1-/- littermates for analysis of microbial populations using 16S rRNA sequencing. Results: Gkn1 mRNA and protein were found only in the stomach and not in any other tissue, confirming the stomach-specific expression of Gln1. Immunohistochemistry revealed the presence of Gln1 in the lumen of the distal gut and the pattern of immunostaining suggested that Gkn1 binds to microbes in the lumen. Gkn1-/- mice were highly susceptible to TNBS induced colitis compared to WT mice, with almost double the lethality 7 days after TNBS treatment. When necropsied 2 days following TNBS treatment, macroscopic examination revealed that Gkn1-/- colons were characterized by watery loose stool, colonic thickening, and significant shortening, compared to WT colons. There were also histologic signs of severe ulceration and inflammation in the treated Gkn1-/- colons compared to WT. Analysis of the microbiome of WT and Gkn1-/- colonic contents revealed no differences in beta-diversity but significantly less observed OTUs in the Gkn1-/- mice compared to WT. There were no differences in either Shannon or Simpson diversity. Comparing the relative abundance of different taxa revealed subtle changes between the communities, notably an increased abundance of Mucispirillum in the Gkn1-/- colonic contents, compared to WT mice. Conclusions: Gln1 is made exclusively in the stomach where it is secreted into the lumen and travels to the distal gut and binds to luminal microbes. Gkn1 is required to protect mice from T-cell mediated colitis and may also modify the distal gut microbiome. This modulation may explain how it protects against T-cell mediated colitis. These results point to a new role for the stomach in protection against IBD, through the production of Gln1 protein.

LONGITUDINAL CHARACTERIZATION OF DYSBIOSIS AND UNCONJUGATED BILE ACID PROFILES IN THE FECES OF DOGS WITH INFLAMMATORY BOWEL DISEASE
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Mucosal evidence suggests that alterations in the microbiota are related to the pathogenesis of inflammatory bowel disease (IBD). However, many of these mechanisms are not well-understood. Bile acids are now increasingly appreciated for their role in diet regulation, and also for their interaction with receptors (i.e., farnesoid X receptor and G protein-coupled receptor), which play a role in intestinal homeostasis and immune regulation. Spontaneously developing IBD in dogs shows many similarities to IBD of humans, including perturbations in gut microbiota and improper activation of the immune system. Therefore, the purpose of this study was to characterize fecal dysbiosis and unconjugated bile acid profiles in dogs with IBD compared to healthy dogs. Fecal samples from 19 CD and 12 UC subjects and 47 healthy controls (HC) were collected. Additionally, 2-6 longitudinal fecal samples were collected from 19 CD and 12 UC subjects. The severity of colitis, as evidenced by reduced body weight loss, colonic shortening and expression of inflammatory cytokines, was assessed at either 2 or 7 days post TNBS administration and assessed for the development of colitis. Result: CD and UC subjects had decreased diversity compared to HC subjects. The abundance of specific bacterial taxa was decreased in CD subjects compared to HC, but increased in UC subjects compared to HC. There were no differences in either Shannon or Simpson diversity. Comparing the relative abundance of different taxa revealed subtle changes between the communities, notably an increased abundance of Mucispirillum in the Gkn1-/- colonic contents, compared to WT mice. Conclusions: Gln1 is made exclusively in the stomach where it is secreted into the lumen and travels to the distal gut and binds to luminal microbes. Gkn1 is required to protect mice from T-cell mediated colitis and may also modify the distal gut microbiome. This modulation may explain how it protects against T-cell mediated colitis. These results point to a new role for the stomach in protection against IBD, through the production of Gln1 protein.

INCREASED AND UNIQUE IMMUNOGLOBULIN TARGETED COMMENSAL BACTERIA IN ACTIVE AND INACTIVE INFLAMMATORY BOWEL DISEASE
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Background: Significant alterations in the microbial community have been described in Ulcerative colitis (UC) and Crohn’s disease (CD) with mucosal immune responses against commensal bacteria. However, the interplay between IBD patients and specific bacteria involved in this process remain unclear. Immunoglobulin A (IgA) is secreted abundantly in the gut mucosa against luminal food and bacterial contents. The specific bacterial targets of this mucosal immunoglobulin during disease can be a read-out for disease eliciting or propagating organisms. In this study, we aimed to understand differences in mucosal immunoglobulin responses against fecal commensal bacteria in active and inactive UC and CD patients as compared to healthy controls. Methods: Fecal samples from 32 CD, 25 UC and 47 healthy controls (HC) were collected. Additionally, 2-6 longitudinal fecal samples were collected from 19 CD and 12 UC subjects. The severity of colitis, as evidenced by reduced body weight loss, colonic shortening and expression of inflammatory cytokines, was assessed at either 2 or 7 days post TNBS administration and assessed for the development of colitis. Results: When compared to HC, both CD (35%±2.5, HC: 1±3%, p<1 *10^-3) and UC (20%±1.8, HC: 1±3%, p<5 *10^-7) subjects had significantly higher IgA targeted commensal bacteria. IgA bound commensal bacterial was only observed in CD (1±2.5%) and UC (1±1.7%) subjects and is not observed HC subjects (0.2% ± 0.00). When patients were analyzed longitudinally, the frequency of IgA-targeted bacteria was directly correlated with the CDAI score (r=0.6, p=0.0001) in CD subjects and with the Mayo score (r=0.6, p= 0.01) in UC subjects. Importantly, total fecal IgA and IgG protein levels did not correlate significantly with the Mayo or CDAI activity scores. Analysis of the IgA bound bacterial repertoire revealed significant differences between IBD patients and healthy controls. There were several taxonomic units (OTUs) that enriched in both disease subtypes but not healthy controls, such as members of the Lachnospiraceae, Clostridiales and Streptococcaceae families. In contrast, the presence of several OTUs, such as members of the Lachnospiraceae and Clostridiales families, was associated with disease resolution. Our study indicates that the dynamic nature of Ig bound bacteria can serve as a non-invasive biomarker for IBD progression. Furthermore, we have identified distinct bacteria that elicited mucosal immune responses in active and inactive UC and CD. This bacteria can be new targets for directed antibiotic or immunomodulatory therapies for the treatment of IBD.