Tu1863

A NOVEL ROLE OF THE STOMACH IN PROTECTION FROM COLITIS

Anne-Marie C. Overstreet, Danika Bakke, Antonia Boger, David Boone

Background: Gastrokine 1 (Gkn1) 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 Gkn1 peptide has been shown to be beneficial to the health of the distal gut. We examined the specificity of Gkn1 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 Gkn1 in the protection from T-cell mediated intestinal inflammation in a mouse model of colitis. **Methods:** Gkn1 protein and mRNA levels were assessed in an array of tissue types from mice. 8 week old WT and Gkn1$^{-/-}$ mice were sensitized to 2,4,6-trinitrobenzensulfonic (TNBS) acid 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 Gkn1$^{-/-}$ 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 Gkn1. Immunodetection showed the presence of Gkn1 in the lumen of the distal gut and the pattern of immunstaining 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:** Gkn1 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 Gkn1 protein.

Tu1864

LONGITUDINAL CHARACTERIZATION OF DYSBIOSIS AND UNCONJUGATED BILE ACID PROFILES IN THE FECES OF DOGS WITH INFLAMMATORY BOWEL DISEASE

Blake C. Guard, Julia B. Honneffer, Michelle M. Jonika, Jonathan A. Lidbury, Jörg M. Steiner, Albert Jergens, Jan S. Suchodolski

Mounting 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 digestion, 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 microbial dysbiosis and fecal unconjugated bile acid dysmetabolism in dogs with IBD upon initial diagnosis and after therapy. Eight dogs that were non-responsive to dietary or antibiotic therapy and had histologically confirmed intestinal inflammation were enrolled at initial diagnosis. Upon initial diagnosis and enrollment, patients received immunosuppressive therapy and a fecal sample was collected to serve as a baseline measurement, and then again at 3 and 8 weeks later. Clinical signs were scored according to the canine IBD activity index (CIBDAI). The canine Dysbiosis Index was evaluated using qPCR, which accounts for Universal 16S rRNA, *Faecalibacterium*, *Turicibacter*, *Streptococcus*, *E. coli*, *Blautia*, *Fusobacterium*, and *Clostridium hiranonis*. Fecal bile acids (i.e., cholic acid, chenodeoxycholic acid, lithocholic acid, deoxycholic acid, and ursodeoxycholic acid) were evaluated by an in-house assay. A Friedman's test was used after testing for normality and a Dunn's post-test used where appropriate. Significance was set at $p<0.05$. The CIBDAI score for patients was decreased in patients 8 weeks after therapy compared to baseline (median score: 0 and 7, respectively). Total secondary bile acids were increased from baseline (median [min-max]: 0.4 µg/mg [0.2-6.7 µg/mg]) to 8 weeks after therapy (median [min-max]: 9.0 µg/mg [3.6-18.1 µg/mg]; p=0.0009). Secondary bile acids expressed as a percent of total bile acids evaluated were significantly increased from baseline (median [min-max]: 16.2 % [3.6-99.6 %]) compared with 8 weeks after therapy (median [min-max]: 97.2 % [73.7-99.3 %]; p=0.0469). There were no significant changes in the Dysbiosis Index from baseline compared to 8 weeks after therapy (p=0.1870). In conclusion, unconjugated bile acid profiles became less dominated by primary bile acids after treatment and shifted to a higher proportion of secondary bile acids. Despite improvement in clinical activity scores, microbial dysbiosis remained present 8 weeks after initiation of therapy. Given potential anti-inflammatory effects of secondary bile acids, further studies are needed to investigate if there is a causal relationship between immunosuppressive therapy and bile acid metabolism.

Tu1865

URSODEOXYCHOLIC ACID AND ITS TAURINE/GLYCINE CONJUGATED SPECIES REDUCE *C.OLI*TOGENIC DYSBIOSIS AND EQUALLY SUPPRESS EXPERIMENTAL COLITIS IN MICE

Lien Van den Bossche, Pieter Hindryckx, Lindsey Devisscher, Sarah Devriese, Sophie Van Welden, Tom Holvoet, Ramiro Vilchez-Vargas, Marius Vital, Dietmar Pieper, Julie Vanden Bussche, Lynn Vanhaecke, Tom Van de Wiele, Martine De Vos, Debby Laukens

Background: The promising results with secondary bile acids in experimental colitis suggest that they may represent an attractive and safe class of drugs for the treatment of inflammatory bowel diseases. However, the exact mechanism by which bile acid therapy confers protection from colitogenesis is currently unknown. Since the gut microbiota plays a crucial role in the pathogenesis of inflammatory bowel disease, and exogenous bile acid administration may affect the community structure of the microbiota, we examined the impact of the secondary bile acid ursodeoxycholic acid (UDCA) and its taurine/glycine conjugates on the fecal microbial community structure during experimental colitis. **Methods:** Acute colitis was induced in mice by administration of 4% dextran sodium sulfate to the drinking water for 7 days. Mice were treated with 500 mg/kg/d UDCA, tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), or placebo by oral gavage. At day 9 of colitis, fecal microbiota profiles were determined through 16S rRNA Illumina MiSeq sequencing and mice were sacrificed at day 10 to assess the severity of inflammation. Ultra-high performance liquid chromatography and high resolution mass spectrometry were performed on fecal samples to analyze the extent of biotransformation of orally administered UDCA, TUDCA and GUDCA. **Results:** Daily administration of UDCA, TUDCA and GUDCA equally lowered the severity of colitis, as evidenced by reduced body weight loss, colonic shortening and expression of inflammatory cytokines. Illumina sequencing demonstrated that bile acid therapy during colitis did not restore fecal bacterial richness and diversity but normalized the colitis-associated increased ratio of *Firmicutes* to *Bacteroidetes*. Interestingly, administration of bile acids prevented the loss of *Clostridium* cluster XIVa and increased the abundance of *Akkermansia muciniphila,* bacterial species known to be particularly decreased in patients with inflammatory bowel disease. Orally administered UDCA, TUDCA and GUDCA were extensively metabolized *in vivo,* resulting in a similar fecal bile acid composition. **Conclusions:** We conclude that UDCA, which is an FDA-approved drug for cholestatic liver disorders, could be an attractive treatment option to reduce dysbiosis and improve inflammation in human inflammatory bowel disease.

Tu1866

INCREASED AND UNIQUE IMMUNOGLOBULIN TARGETED COMMENSAL BACTERIA IN ACTIVE AND INACTIVE INFLAMMATORY BOWEL DISEASE

Sunaina Khandelwal, Emily Vivio, Rodney D. Newberry, Miles Parkes, Matthew A. Ciorba, Chyi-Song Hsieh

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 gut microbiota being key drivers of IBD pathogenesis. However, the 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. Ig responses generated against fecal commensal bacteria were studied using flow cytometric sorting coupled with16s rDNA sequencing. **Results:** When compared to HC, both CD (CD: 35%±2.5, HC: 13±1.5%, $p<5*10^{-6}$) and UC (UC: 20%±1.8, HC: 13±1.5%, $p<5*10^{-2}$) subjects had significantly higher IgA targeted commensal bacteria. IgG bound commensal bacteria was only observed in CD (15±1.2%) and UC (16%±1.7) subjects and is not observed HC subjects (0.2% ± 0.00). When patients were analyzed longitudinally, the frequency of Ig-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 operational taxonomic units (OTUs) that were enriched in both disease subtypes but not healthy controls, such as members of the Lachnospiraceae, Clostridiales and Streptococcaceae families. We also observed a dynamic IgA binding profile of certain bacterial OTUs. For example, Streptococcus species and several clostridia species showed IgA-binding only in active UC subjects. Similarly, in CD subjects, Lachnospiraceae and Clostridiales species lost IgA binding upon resolution of the disease measured by CDAI score and Enterobacteriaceae, Lactobacillaceae, and others remained IgA coated regardless of disease activity. **Conclusions:** 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 elicit mucosal immune responses in active and inactive UC and CD. These bacteria can be new targets for directed antibiotic or immunomodulatory therapies for the treatment of IBD.

Tu1867

FIRST ANALYSIS FROM UK IBD TWIN BIOBANK: 16S RRNA GENE SEQUENCING IDENTIFIES REDUCED DIVERSITY IN IBD AND BACTERIAL TAXA ASSOCIATED WITH DISEASE

Hannah Gordon, Julie A. McDonald, Timothy Orchard, Julian Marchesi, Marcus Harbord, William A. Blad

Background Previous studies have shown that the gut microbiota plays an important role in IBD. However there is no consensus on which bacteria are responsible for the disease. 16S gene profiling studies generate large amounts of information, but are confounded by genetic and environmental factors. Twin studies are instrumental in controlling these