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Chronic Colitis in *Bacteroides Thetaiotaomicron*-Monoassociated HLA-B27 Transgenic Rats is Associated With Altered Transcription of Receptor and Metabolic Genes in Luminal Bacteria

Jonathan J. Hansen, Yong Huang, Daniel A. Peterson, Jeffrey I. Gordon, Eugene B. Chang, Ryan B. Sartor

Background: Current evidence indicates that inflammatory bowel diseases (IBD) may be caused in part by aberrant immune responses to commensal intestinal microbes including *Bacteroides thetaiotaomicron* (*B. theta*). Healthy, germ-free HLA-B27 transgenic (Tg) rats develop chronic colitis when colonized with complex gut commensal bacteria or *Bacteroides vulgatus* whereas non-transgenic (nTg) rats remain disease-free. However, the role of *B. theta*, a well-characterized anaerobic commensal bacterium, in causing disease in Tg rats is unknown. Moreover, while host immune responses to the gut microbiota have been extensively studied, relatively little is known about how microbes respond to host inflammation. **Hypothesis**: *B. theta* monoassociated Tg rats develop chronic, immune-mediated colitis that is associated with transcriptional responses in the bacteria that initiate and perpetuate disease. **Methods**: Four Tg and five nTg rats were monoassociated with a human isolate of *B. theta* (VPI-5482) for six weeks. Colonic inflammation was quantified by blinded histological scoring and real-time RT-PCR assays of pro-inflammatory cytokines. Cecal bacterial concentrations were measured by quantitative plating on BHI agar. Whole genome transcriptional profiling of *B. theta* recovered from the cecum was performed using custom GeneChips and data analyzed using DChip, Significance Analysis of Microarrays, and Gene Set Enrichment Analysis (GSEA) software. **Results**: *B. theta* monoassociated Tg rats had significantly more colonic inflammation and increased colonic levels of pro-inflammatory cytokine mRNAs than nTg controls (Table). Transcriptional profiles of cecal *B. theta* were significantly different in Tg vs. nTg rats (329 transcripts representing 44 KEGG canonical pathways). GSEA revealed that the GO molecular function of receptor activity, which is comprised mainly of genes that encode nutrient binding proteins, was significantly enriched with genes upregulated in *B. theta* from Tg rats [GSEA false discovery rate (FDR)=0.148]. KEGG canonical pathways of ribosome (FDR=0.048), oxidative phosphorylation (FDR=0.098), pyrimidine metabolism (FDR=0.081), purine metabolism (FDR=0.197), peptidoglycan biosynthesis (FDR=0.184), and metabolism (FDR=0.191) were significantly enriched with genes downregulated in *B. theta* from Tg rats. Numbers of viable bacteria/gram cecal contents in Tg vs. nTg rats were not significantly different. **Conclusions**: A well-characterized human isolate of *B. theta* induces mild colitis in HLA-B27 Tg rats and colitis is associated with changes in the expression of microbial metabolic and nutrient binding pathways, but no difference in concentrations of viable cecal bacteria. Mechanistic studies of differentially expressed *B. theta* genes may reveal novel pathways that initiate and/or perpetuate IBD.

	Mean Cecal Histology Score	Mean Distal Colon Histology Score	Mean Cecal IFNγ mRNA Relative $x10^{-5}$	Mean Distal Colon IFNγ mRNA Relative $x10^{-5}$	Mean Cecal TNF mRNA Relative $x10^{-4}$	Mean Distal Colon TNF mRNA Relative $x10^{-4}$
Tg	1.56	1.06	2.3	5.0	6.5	5.6
nTg	0.37	0.35	1.5	1.9	2.7	2.6
p value	2×10^{-6}	9×10^{-5}	0.013	0.002	5×10^{-4}	0.001

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IL-6 is Required for Microbiome-Mediated and Innate Immune-Dependent Recruitment of Neutrophils and Macrophages and is Necessary for Mucosal Repair After Intestinal Injury

Di Meng, Weishu Zhu, N. Nanda Nanthakumar

Commensal bacteria are critical for maintaining normal intestinal homeostasis and play an important role in rapid recovery after mucosal injury in the gut. They also play a major role in the onset of inflammatory bowel disease (IBD), however, the molecular and cellular mechanisms by which gut microbiome contribute to intestinal homeostasis and how they modulate the intestinal innate immune responses are unknown. Acute intestinal injury created by specific chemical irritants ————- (DSS), ———————— (TNBS) or ———————— (OAA) in mice fully recover by eliciting either innate, Th1 or Th2 responses, respectively. The conventionally raised mice were able to recover from mucosal injury caused by DSS, TNBS and OAA. However, the recovery was significantly compromised in bacteria depleted (BD) mice after DSS-induced, but not by TNBS- or OAA-induced mucosal injury. The morbidity and mortality (40%) observed in DSS-treated BD mice were associated with compromised recruitment of neutrophils and macrophages to the site of injury. These results indicate that commensal bacteria were required for innate immune-mediated mucosal repair and to reduce the mortality caused by DSS-induced injury in BD mice. Similar results were observed in wild-type BD mice or BD SCID mice indicating no role in adaptive immune system in the mucosal recovery after DSS-induced injury. We then investigated which subset of commensal bacteria were responsible for successful recovery from DSS-induce mucosal injury. The Gram (-) bacteria (B. fragilis and *E. coli*) or lipoplysaccharide (TLR4 ligand) alone was sufficient to rescue the morbidity and mortality associated with DSS-induced injury in BD mice. Both Gram (+) bacteria or peptidoglycans (TLR2 ligand) were unable to rescue these mice. Similar morbidity and mortality was observed in TLR4-/- and MyD88-/- mice after DSS treatment. We assayed for a number of cytokines and chemokines induced by commensal bacteria after injury in the colon. We identified that mucosal induction of MIP2 and IL6 were significantly compromised in DSS-treated BD mice. Exogenous MIP2 was sufficient to rescue BD mice from DSS-induced injury and to restore neutrophil and macrophage recruitment to the site of injury. The ability to repair the mucosa after DSS induced injury was lost in IL-6-/- mice. However, exogenous MIP2 was able to rescue IL-6-/- mice suggesting that IL-6 is upstream of MIP2 signaling necessary for neutrophil and macrophage recruitment or recovery. This suggests a previously unknown role of TLR4/MyD88-dependent IL6 and MIP2 induction which is needed for restoring mucosal integrity after acute intestinal injury.

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Temporary Controllable Pseudobezoars: Non-Invasive Alternative to Surgical Gastric Volume Reduction for the Treatment of Obesity

Marlen G. Deneva, Anguel Marintchev, Orly Yadid-Pecht, Michel Fattouche, Robert C. Bray, Martin P. Mintchev

Background. Recently developed temporary, controllable pseudobezoar therapy provides an obesity treatment approach based on a non-invasive, dynamic, long term sustainable gastric volume reduction from inside the stomach. This paper aims at investigating the impact of temporary controllable intragastric pseudobezoars in a blind, placebo-controlled, crossover study in healthy volunteers. Methods. Commercially available pseudobezoar product (Zalak B, EatLittle Inc, Calgary, Canada) was tested. A total of 16 healthy but overweight and/or obese subjects (3M, 13F, mean age 44.0 ± 12.0 years, mean body weight 93.8 ± 21.9 kg, BMI 33.3 ± 5.6 kg/m2, mean waist 103.3 ±13.8 cm, mean hip 114.3 ±12.3 cm) participated in a blind, placebo-controlled, cross-over chronic trial for a period of two months. Two groups of 8 participants were randomly formed, denoted as Group A (starting with the pseudobezoar product, 8 F) and Group B (starting on placebo, 3M, 5F). The administration during the entire study period included the trans-oral intake of 2 pseudobezoar or placebo capsules 15 to 30 min before principal meals with at least 300 ml of room temperature water, 3 times a day. Group A started on the pseudobezoar product for the first 30 days, while Group B was put on placebo. After the first month the groups were crossed-over for another month of study. In parallel, all subjects were provided with dietary recommendations. Body weight (BW), waist circumference (WC), hip circumference (HC), Haber satiety scale scores and side effects were monitored and recorded. Results. The one-month pseudobezoar treatment of all participants resulted in a statistically significant increase in satiety (Haber scores 4.2+/-1.3 vs. 3.8+/-1.1 in placebo, $p \leq 0.05$) and greater reduction in BW (3.5% vs. 2.6%), BMI (3.5% vs. 2.7%), and WC (3.4% vs. 2.4%) compared to placebo ($p \leq 0.01$). After the first month, Group A produced greater reduction in BW (3.3% vs. 1.9%), BMI (3.3% vs. 2.0%), and WC (2.6% vs. 1.7%). After switching, the pseudobezoar therapy month for Group B resulted in BW, BMI, WC and HC reductions of 3.7%, 3.5%, 4.2% and 1.3% respectively, while Group A (switched to placebo) did not regain weight and continued its weight loss with an additional 3.3%. At the end of the pseudobezoar therapy 31.3% of the volunteers had more than 3% BMI reduction (vs 18.7% on placebo) and 25% registered more than 5% BMI reduction (vs 12.5% on placebo, achieved in the post-pseudobezoar therapy month for Group A only). The pseudobezoar therapy was generally well tolerated. The most commonly reported, but not significant side effects were heaviness in the stomach and bloating. Conclusion. Intragastric temporary controllable pseudobezoars have the potential of becoming a safe non-invasive alternative to bariatric surgery. Optimization of the pseudobezoar implements can increase its long-term effectiveness.

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Sleeve Gastrectomy in Obese Mice Results in Elevated Serum Bile Acids and Reduced Hepatic Steatosis That Correlate With Weight Loss Post Surgery

Andriy Myronovych, Michelle Kirby, Randy J. Seeley, Rohit Kohli

Recently patients who have had bariatric surgery such as Roux-en-Y-Gastric Bypass (RYGB) and vertical sleeve gastrectomy (VSG) have been shown to have increased serum bile acid levels and reduced hepatic steatosis in addition to a decrease in body weight. Our aim was to investigate the effect of VSG on non-alcoholic fatty liver disease (NAFLD) in obese mice and evaluate its impact on serum bile acid levels. Methods: To induce obesity adult male C57Bl/6 mice were fed a saturated high-fat diet (HFD, 58 kcal%) for three weeks. The mice were then randomized to receive either Sham operation (SH) (n=5) or VSG surgery (n=11). Mice were kept on liquid diet for the first three days after surgery and afterwards placed back on the same HFD. Animals were sacrificed four weeks post-surgery. Results: All mice lost weight after the surgery, however, VSG mice lost greater weight compared to the SH animals (-5.73 ± 1.42 vs. -1.79 ± 0.51g; $p < 0.0001$). Starting from the post-operative day 3 a significant difference in body weight was observed between the VSG and SH groups. All mice gradually began re-gaining weight but VSG mice gained weight at a slower rate. VSG mice has lower body weight compared to SH operated mice even at sacrifice (28.4 ± 1.5g vs. 32.64 ± 1.91g VSG and SH respectively; $p<0.001$). Food and water intake was not different between the groups. Plasma fasting glucose and serum alanine amino transferase (ALT) levels were not different between the SH and VSG groups at sacrifice 4 weeks post-surgery. Oil-red-O staining on frozen liver sections showed decreased neutral lipid accumulation in VSG compared to SH mice. Hepatic triglycerides were significantly lower in VSG (37.1 ± 7.7) compared to SH livers (52.53 ± 15.36 mg/dL/100mg wet liver; $p= 0.02$). A significant correlation was observed in VSG mice between liver triglyceride content and body weight at sacrifice at 4 weeks post-surgery ($p=0.005$; $R2= 0.69$). Further, in VSG mice at sacrifice a strong correlation was also observed between serum total bile acid levels and body weight change ($p=0.013$, $R2=0.74$). Conclusion: Mice having VSG lost more body weight and had reduced triglyceride accumulation in the liver. The change in weight post-VSG surgery strongly correlated with liver triglyceride content and increased serum total bile acid levels. These results suggest a potential role of bile acids in the metabolic improvements after VSG in mice including reduction of hepatic steatosis. VSG in diet induced obese mice provides a novel and relevant tool to investigate the role of bile acids in weight loss and NAFLD improvement seen after bariatric surgical procedures.

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Novel Action of Pegylated Arginine Deiminase to Protect Against Colitis

Helieh S. Oz, Jian Zhong, Willem J. de Villiers

Background: Arginine deiminase (ADI), an arginine metabolizing enzyme involved in cell signaling, is dysregulated in multiple inflammatory diseases and cancers. Microbial derived ADI (5h half life) when covalently bound to polyethylene glycol (PEG) (ADI-PEG) has an increased life span of 6 days in the blood circulation. We hypothesized that pegylated ADI (ADI-PEG) to provide protection against colitis. Methods: Dextran sodium sulfate colitis was induced in IL-10-deficient and BALB/c (WT) mice. Animals were monitored for symptoms of