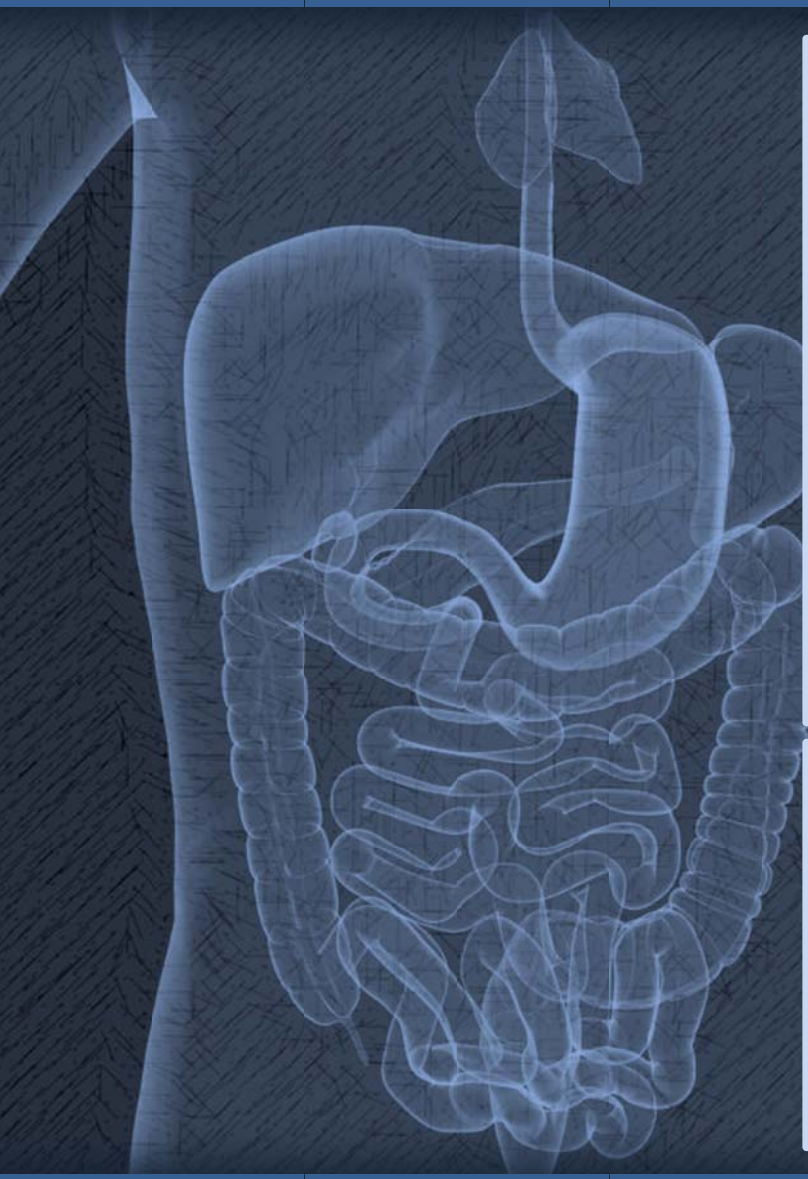
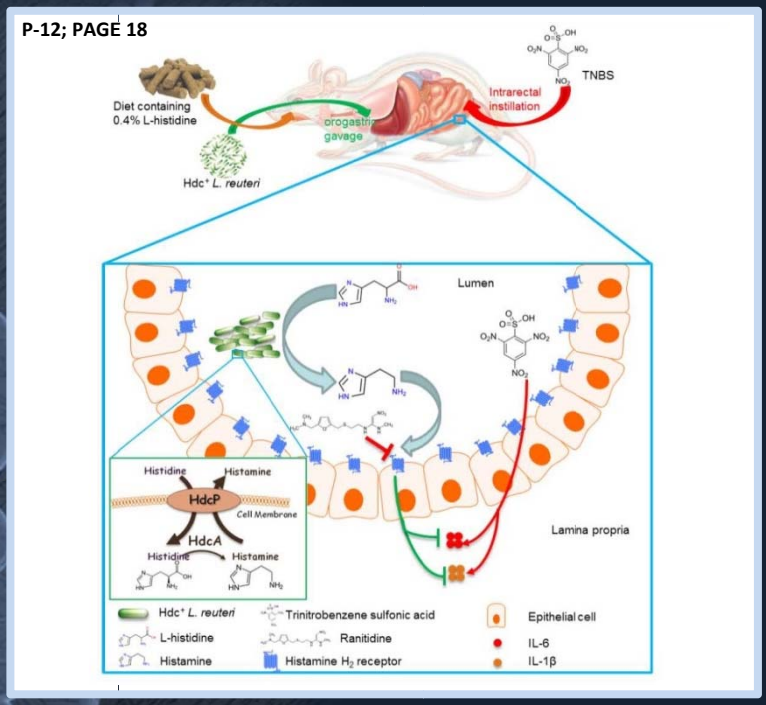
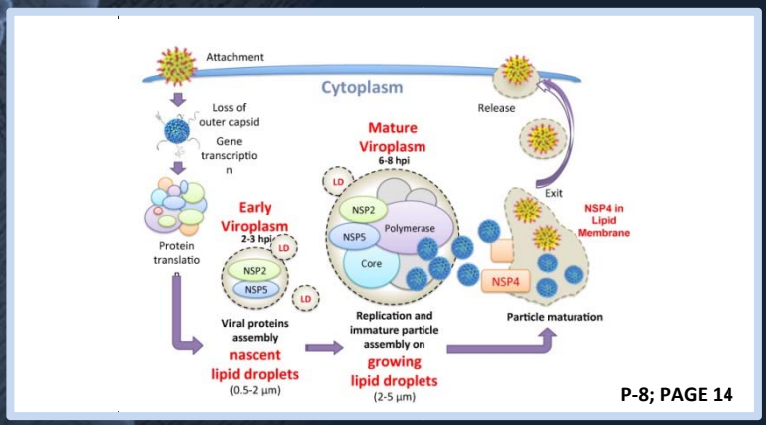
Texas Medical Center Digestive Diseases Center
6th Annual Frontiers in Digestive Diseases Symposium

"Frontiers of Inflammatory Bowel Diseases in 2015"







Saturday, February 7, 2015

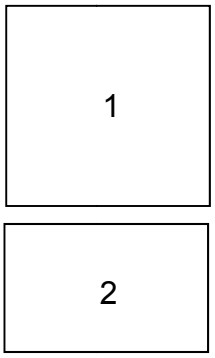
Baylor College of Medicine

Houston, Texas

Texas Medical Center Digestive Diseases Center
6th Annual Frontiers in Digestive Diseases Symposium:
Frontiers of Inflammatory Bowel Diseases in 2015

Saturday, February 7, 2015
Baylor College of Medicine – Cullen Auditorium
One Baylor Plaza, Houston, Texas 77030

On the cover:



(1) P-12. Chunxu Gao. Intrarectal instillation of trinitrobenzene sulfonic acid (TNBS) induced colitis as indicated by increased pro-inflammatory cytokine gene (IL-6, IL-1β) expression in the colon. When mice were fed with histidine-containing diet, Hdc+ *L. reuteri* administered to mice by orogastric gavage converts L-histidine to histamine by histidine decarboxylase (HdcA) and exports histamine to the lumen by histidine/histamine antiporter (HdcP). *L. reuteri* derived histamine activates histamine H2 receptor (H2R) on epithelial cells and triggers the anti-inflammatory signaling pathway as indicated by suppression of pro-inflammatory cytokine gene (IL-6, IL-1β) expression. H2R specific antagonist (ranitidine) may block this anti-inflammatory effect.

(2) P-8. Jeanette Criglar. Lipid droplets (LDs) are required for RV replication. RV proteins NSP2 and NSP5 are required for nascent viroplasm formation. Newly formed viroplasms co-localize with LDs. Small viroplasm/LDs merge to make larger viroplasm/LDs. After accumulating other virus proteins, mature viroplasms associate with NSP4-containing membranes for infectious particle assembly prior to cell exit.

Texas Medical Center Digestive Diseases Center
6th Annual Frontiers in Digestive Diseases Symposium:
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A G E N D A
Saturday, February 7, 2015
Baylor College of Medicine – Cullen Auditorium
One Baylor Plaza, Houston, Texas 77030

7:30-8:00 AM	**Registration and Breakfast**
8:00-8:15 AM	**Welcome and Opening** HASHEM EL-SERAG, M.D., M.P.H. RICHARD KELLERMAYER, M.D., PH.D.
8:15-9:00 AM	**"Inflammatory Bowel Diseases, Current Challenges at the Bedside"** MANREET KAUR, M.D.
9:00-9:45 AM	**"Genetics of Inflammatory Bowel Diseases: Therapeutic Considerations"** ALEIXO MUISE M.D., PH.D., FRCPC
9:45-10:05 AM	**Coffee break**
10:05- 10:50 AM	**"New Insights into Epithelial Metabolism and Inflammatory Bowel Diseases"** SEAN COLGAN, PH.D.
10:50-11:35 AM	**"Mucosal Immunology in Inflammatory Bowel Diseases"** CHARLES ELSON, M.D.
11:35-12:45 PM	**Poster Viewing** Boxed Lunches Provided
12:45-1:15 PM	**Poster Presentations by Selected Recipients** 5 minute presentations followed by 2 minute question-answer
1:15-2:00 PM	**"The Microbiome of Inflammatory Bowel Diseases: Nutritional Impact and Therapeutic Considerations"** GARY WU, M.D.
2:00-2:45 PM	**"From Genes and Microbes to the Bedside of Inflammatory Bowel Disease in 2015"** HARLAND WINTER, M.D.
2:45-3:00 PM	**Closure and Acknowledgements** HASHEM EL-SERAG, M.D., M.P.H. JAMES VERSALOVIC, M.D., PH.D.

Texas Medical Center Digestive Diseases Center
6th Annual Frontiers in Digestive Diseases Symposium:
Frontiers of Inflammatory Bowel Diseases in 2015

Texas Children's Hospital®

APPROVED CME ACTIVITY

Hosted by Texas Medical Center Digestive Disease Center
Sponsored by Texas Children's Hospital
Saturday, February 7, 2015 | 8:00 am – 3:00pm | BCM Cullen Auditorium

MANREET KAUR, M.D.
Assistant Professor, Baylor College of Medicine

ALEIXO MUISE M.D., PH.D., FRCPC
Co-Director, Inflammatory Bowel Disease Centre, The Hospital for Sick Children, Toronto

SEAN COLGAN, PH.D.
Director of Mucosal Inflammation Program, Vice Chair for Research, University of Colorado School of Medicine

CHARLES ELSON, M.D.
Professor, UAB School of Medicine

GARY WU, M.D.
Ferdinand G. Weisbrod Professor in Gastroenterology, Perelman School of Medicine University of Pennsylvania

HARLAND WINTER, M.D.
Director, Pediatric Inflammatory Bowel Disease Center, Massachusetts General Hospital

Educational Objectives: At the conclusion of the lecture, the participants should be better able to:

- Define the genetic risk factors of IBD and interpret those in clinical practice.
- Define the role of the microbiome in IBD and how nutritional interventions may impact that.
- Define the immune mechanisms of IBD and how current and future therapies modify those.
- Interpret the role of epithelial pathology in disease development.

Target Audience: Physicians, residents, fellows, and other healthcare professionals

Accreditation/Credit Designation:
This live activity has been planned and implemented in accordance with the accreditation requirements and policies of the Texas Medical Association through the joint providership of Texas Children's Hospital and Texas Medical Center Digestive Diseases Center. Texas Children's Hospital is accredited by the TMA to provide continuing medical education for physicians.

Texas Children's Hospital designates this live activity for a maximum of **4.5** ***AMA PRA Category 1 Credit™.*** Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Disclosure:
Drs. Kaur, Muise, Colgan, Elson, Wu, and Winter have reported no relationships with proprietary entities related to the content of this activity. Persons involved in the planning of this activity have reported no relevant financial relationships with any commercial interest.

Statement of Commercial Support:
No commercial support was used in this activity.

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Amelioration of Experimental NEC by Surfactant Protein-A

*Weizhen Bi, †Nicole Y. Fatheree, †#Yuying Liu, §Constance L. Atkins, #Sam C. Flanders, #Alen K. Michael and *#Joseph L. Alcorn
*Division of Neonatal-Perinatal Medicine, †Division of Gastroenterology, §Division of Pulmonary Medicine, #Pediatric Research Center, Department of Pediatrics The University of Texas Medical School at Houston, Houston TX

Necrotizing enterocolitis (NEC) results in the destruction of all or part of the bowel, and is one of the most feared complications among prematurely-born infants. Risk factors for developing NEC include ingestion of infant formula, intestinal hypoxia, and intestinal mucosa immaturity. Pathogenesis of NEC involves exposure to lipopolysaccharide (LPS), increased levels of inflammatory cytokines and enhanced activity of toll-like receptor 4 (TLR4) in premature intestine. Other than surgery, effective treatments after onset of NEC are lacking.

Surfactant protein A (SP-A) plays a key role in innate immunity; SP-A modulates inflammation, dampens TLR4 activity and binds LPS. We have reported that gavage of purified human SP-A (5 µg/day) significantly decreased mortality and the incidence of NEC in an experimental rat pup model of NEC. SP-A also reduced intestinal TLR4 and inflammatory cytokine levels. Our goal was to demonstrate a dose-effect relationship between SP-A and NEC and to determine the efficacy of SP-A in established intestinal inflammation in the model. These results may define the dose of purified SP-A that is most effective in amelioration of NEC and determine if administration of SP-A could be used therapeutically in established NEC.

Rat pups (3 day old) were separated from dams, separated into groups and fed formula. Experimental NEC was induced by thrice daily exposure to 10 min of hypoxia (5% O_2, 95% N_2). Various amounts of SP-A was administered in the formula during the initial daily feeding or when dictated. On the fourth day, pups were sacrificed and ileum harvested for assessment of NEC and measure of inflammatory cytokines by ELISA.

Unlike previously used regimes to induce experimental NEC in this model, results indicated that hypoxia treatment of pups did not result in robust assessment of NEC compared to untreated pups. However, changes elicited by administering various amounts of SP-A were observed. When <5 µg of SP-A was administered daily, little effect on assessment of NEC and IL-1β levels was observed. When ≥5 µg of SP-A was administered, NEC was decreased; however, a dose-dependent effect was not observed. On the other hand, SP-A appeared to have a dose-dependent effect to decrease intestinal IL-1β levels. Delayed administration of SP-A (5 µg) provided very interesting results; the incidence of NEC was reduced as were the levels of IL-1β when SP-A was administered concurrently with treatment to induce NEC or 1 day after the initial treatment. On the other hand, administration of SP-A 2 days after treatment to induce NEC had little effect on assessment of NEC or IL-1β levels. These results suggest that while a threshold level of SP-A must be used to ameliorate NEC, the effect of SP-A is not dose-dependent. In addition, there is a window of opportunity to administer SP-A to reduce NEC in the initial stages of gastric distress. However, the beneficial effects of SP-A are not observed in advanced stages of distress.

These results suggest that in addition to its prophylactic use, SP-A can be used to ameliorate acute NEC in its initial stages.

Lactobacillus reuteri DSM 17938 suppresses T_H17 cell differentiation through orphan receptor RORα/γ

Baokun He[1,3], Yuying Liu[1,3], Thomas K. Hoang[1,3], Dat Q. Tran[2,3], and J. Marc Rhoads[1,3]
Department of Pediatrics, Divisions of [1]Gastroenterology and [2]Allergy/Immunology/Rheumatology, [3]Pediatric Research Center, University of Texas Health Science Center at Houston Medical School, Houston, TX 77030, USA

Background: $CD4^+$T helper lymphocytes that express interleukin-17 (T_H17 cells) play a pathogenic role in multiple inflammatory diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). The discovery of T_H17 cells provides an attractive target for therapeutic application aimed at inhibiting the function of these cells. The retinoic-acid-receptor-related orphan receptors α (RORα) and γ (RORγ) are required for the full differentiation of naïve $CD4^+$T cells into T_H17 cells, and for induction of IL-17 transcription. Therefore, an attractive strategy for the development of novel therapeutics to treat T_H17-mediated inflammatory diseases is to inhibit the activity of RORα and RORγ. Anti-inflammatory effects of probiotic *Lactobacillus reuteri* DSM17938 (LR17938) have been shown in 3 animal models of inflammation: lipopolysaccharide (LPS)-induced intestinal inflammation necrotizing enterocolitis (NEC), and $Foxp3^+$regulatory T (Treg)-deficient Scurfy mice. How LR17938 affects T_H17 responses in inflammatory diseases remains unclear. We hypothesized that the beneficial effects of LR17938 in inflammatory diseases may be partially dependent on inhibition of T_H17 cell differentiation through suppressing the activities of RORα and RORγ.

Aims: To determine if LR17938 suppresses T_H17 cell differentiation through targeting RORα and RORγ.

Methods: For T_H17 differentiation, naïve $CD4^+$T cells isolated from the spleen of C57BL/6J mice were incubated for 5d in T_H17 cell differentiation medium in the presence or absence of LR17938 (ratio of 1:1 (LR: cell)), re-stimulated by PMA/inomycin with brefeldin for 5h, and then stained for CD4 and intracellular IL-17A to be analyzed by flow cytometry. A cell-based RORα or RORγ-dependent reporter assay was used to identify the effects of LR17938 on transcriptional activity of RORα and RORγ, in which the DNA binding domain of GAL4 was fused to the ligand binding domain (LBD) of mouse RORα or RORγ. We also co-transfected mouse RORα or RORγ full length and IL-17A promoter reporter construct with RORα/γ response element (RORE) into EL4 cells to determine the effect of LR17938 on IL-17A promoter activity. The expression of T_H17 signature genes, including IL17A, IL17F and other RORα/γ target genes, in EL4 and T_H17 cells was quantified by real-time PCR.

Results: We found that LR17938 inhibited mouse T_H17 cell differentiation *in vitro*. The percentage of IL17A-producing $CD4^+$T cells (T_H17) in T_H17 cell differentiation medium was significantly increased compared to T_H0 in which when naïve $CD4^+$T cells were stimulated in a medium containing anti-CD3/CD28 but without the components for T_H17 cell differentiation. LR17938 treatment significantly decreased the percentage of IL17A-producing T_H17 cells in the T_H17 differentiation assay. Subsequently, we assessed the effect of LR17938 on transcriptional activity. We observed that LR17938 inhibited RORα or RORγ-dependent reporter activity in a dose-dependent manner. LR17938 also strongly inhibited IL-17 promoter activity in EL4 cells overexpressing RORα or RORγ, which was confirmed by real-time PCR assays.

Conclusions: Probiotic LR17938 suppresses T_H17 cell differentiation and function via inhibiting RORα/γ activity. LR17938 may be helpful in treating T_H17-mediated inflammatory diseases.

The association of Krüpple-like factor 5 and mitogen-activated protein kinase signaling in normal intestinal proliferation.

Kristin N. Bell[1] and Noah Shroyer[1, 2]
[1]Graduate Program in Molecular and Developmental Biology, University of Cincinnati, Cincinnati, OH; [2]Department of Medicine - Division of Gastroenterology, Baylor College of Medicine, Houston, TX.

Background and Aims: In the adult intestine, all proliferative stem and progenitor cells are located in the crypts of Lieberkühn. Within the intestinal crypts expression of the transcription factor Klf5 is confined to proliferating cells. Homeostatic maintenance of these stem and progenitor cell populations is essential to support the rapid turnover of the intestinal epithelium. We have previously shown that Klf5 is required for cellular proliferation to maintain intestinal homeostasis and for stem cell maintenance; however, the mechanism by which this works remains unclear. KRAS and HRAS mutations play a known oncogenic role in intestinal cancers. Loss of one Klf5 allele ameliorates the tumorigenic effects of this signaling pathway in this context. Furthermore, *in vitro* cell culture studies suggest that Klf5 is required for MAPK signaling. Given these results, we hypothesize that MAPK signaling is required for Klf5's proproliferative nature in normal intestinal homeostasis.

Methods: To determine if Klf5 requires MAPK signaling for proliferation within the intestine, an inducible Klf5-floxed allele was combined with constitutively active KRAS. Simultaneous recombination of Klf5 and activation of KRAS were driven by Villin-creER in mice. Tamoxifen was administered for five consecutive days prior to sacrifice on the subsequent day. Tissue was analyzed using conventional immunohistochemistry, western blot analysis, and RT-qPCR. Functional stem cell assays were conducted *in vitro* using three-dimensional enteroids derived from jejunal crypts.

Results: In addition to decreased proliferation within the crypts of the intestine, loss of Klf5 coincided with a loss of phosphorylated MEK and ERK. Conversely, immunohistological analysis showed that increased Klf5 lead to misexpression and increase of phosphorylated ERK. Expression of KRAS *in vivo*, rescued the loss of proliferation. Additionally, enteroids isolated from recombined Klf5 crypts failed to from the characteristic budding structure of their wildtype counterparts. Under these same conditions, reexpression of KRAS was able to rescue their formation. However, these enteroids eventually die like those Klf5 KO enteroids. RT-qPCR confirmed that stem cell loss was maintained after reexpression of KRAS in these mice.

Conclusions: Data gathered from our rescue experiments suggest that MAPK signaling may be downstream of Klf5. However, results from these experiments indicate, that while Klf5 is necessary for stem cell maintenance and normal proliferation, MAPK signaling is only sufficient to restore normal progenitor proliferation rather than stem cell numbers. Preliminary results from this study suggest a role for Klf5 in differential signaling pathways for stem and progenitor cells. To date it is unclear what the mechanism of Klf5 or the role of MAPK signaling is in the normal intestine, but given their known role intestinal disease, specifically cancer, it is important to discern their integrative nature in normal homeostasis. Future experiments will be required to determine the specific regulatory effects of Klf5 and how this may integrate with other known proliferative pathways within the intestine.

Human Intestinal Enteroid Cultures: A New Functional Model of Gastrointestinal Virus Infection

Blutt SE[1], Saxena K[1], Ettayebi K[1], Zeng X[1], Broughman J[1], Crawford SE[1], Karandikar U[1], Conner ME[1],Foulke-Abel J[2], In J[2], Kovbasnjuk O[2], Zachos NC[2], Donowitz M[2], and Estes MK[1]
1. Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX
2. Department of Medicine, Gastroenterology Division, Johns Hopkins University School of Medicine, Baltimore, MD; Corresponding Author: Estes MK, Dept. of MolViro and Micro, Baylor College of Medicine

A significant limitation in translational research in the gastrointestinal tract is the absence of reliable pre-clinical models that recapitulate human physiology and disease pathology. We have established models of the small intestinal epithelium using advancements in stem cell biology pioneered by the Clever's Lab (Hubrecht Institute). Human intestinal enteroids (HIEs) self-organize into villus-crypt structures, which contain the normal complement of intestinal epithelial cell types (enterocytes, goblet, enteroendocrine, and Paneth cells) and are physiologically active based on responses to agonists. We have characterized these new, non-transformed, 3D HIE cultures as pre-clinical models of human rotavirus (HRV) infection, which kills nearly 500,000 children annually. Studies on HRVs have been limited as they are difficult to culture in transformed cell lines and do not infect small animals. RVs generally exhibit host range restriction in replication. Thus, HRV does not replicate in mice whereas mouse RV and some other animal RVs (ARV) exhibit a broader host range and do replicate in mice.

In order to determine whether RVs exhibit host range restriction in HIEs, we inoculated differentiated and undifferentiated HIEs from several patients with either HRVs or ARVs from a bank of HIEs (N=52) derived from patient small intestine biopsies or surgical tissues. We quantitated the (1) amount of viral RNA by qRT-PCR, (2) number of infected cells using flow cytometry and immunofluorescence confocal microscopy, and (3) yield of virus by fluorescent focus assay. HRVs infected more cells within HIEs than ARV (50% compared to 13%). Undifferentiated HIEs, consisting primarily of immature enterocytes and stem cells, were less susceptible to infection compared to fully differentiated HIEs that consist predominately of mature enterocytes (12.6% compared to 37.6%). Like HRV, infection with ARV was also higher in differentiated HIEs compared to undifferentiated HIEs (13% compared to 8%). Electron microscopy and immunofluorescence studies validated that HRV-infected HIEs show classical features of RV-infected cells, including presence of virus factories (viroplasms) and induction of lipid droplets. HRV causes diarrhea by several mechanisms including modulating fluid flow through increased apical chloride channel activity. We investigated whether HRV or the RV enterotoxin, NSP4, cause physiological changes in HIEs. Through time-lapse microscopy, we observed that both HRV infection and NSP4 treatment of HIEs induced luminal swelling indicative of increased fluid secretion.

These findings establish HIEs as new models to understand host physiology, disease pathophysiology and the intestinal epithelial response, including host restriction to gastrointestinal infections such as HRV infection. HIEs allow us to address new questions about human host-pathogen interactions such innate immune responses, stem cell activity, cell-cell communication within the epithelium in response to infection as well as to identify and test new drug therapies to prevent/treat diarrheal disease. In addition, HIEs provide a physiologically relevant model system to examine the role of the microbiota in altering infection or drug treatment.

Funding: National Institutes of Health Grants U18-TR000552, R01-AI080656, P30-DK056338 (Baylor), and P30-DK089502 (Hopkins) and Howard Hughes Medical Research Fellowship 57007689.

Gfi1 functions as a potential tumor suppressor by inducing apoptosis in colorectal cancer cells

Min-Shan Chen[1], Noah F Shroyer[1,2]

[1.] Integrative Molecular and Biomedical Sciences Graduate Program, Baylor College of Medicine, Houston, Texas, USA, [2.] Division of Medicine, Section of Gastroenterology & Hepatology, Baylor College of Medicine, Houston, Texas, USA

Colorectal cancer (CRC) is the third most common and the third leading cause of cancer death in the United States. The growth factor-independent 1 (Gfi1) zinc finger is a transcription factor repressor implicated in the differentiation of secretory precursors into goblet and Paneth cells in the intestinal epithelium. Previous studies suggested that gain or loss of function of Gfi1 leads to cancer, such as leukemia and neutropenia. However, the role of Gfi1 in CRC is still largely unknown. Recent genome-wide association studies from The Cancer Genome Atlas have revealed that the gene expression of Gfi1 is associated with less tumor aggressiveness in CRC, indicating Gfi1 has a role in tumor development in CRC. Therefore, we hypothesize that Gfi1 acts as a tumor suppressor of CRC tumorgenesis. In our work, we find that malignant CRC cells display weaker Gfi1 staining compared to normal tissue in human colorectal specimens. Moreover, transient expression of Gfi1 in human colorectal cells results in increased apoptosis. Future directions will focus on examining the role of Gfi1 in CRC in Apc^{Min} mouse model *in vivo*. We expect that conditional deletion of Gfi1 in intestinal epithelium will results in worse outcome. In the contrast, re-expression of Gfi1 in intestinal tumor may prevent tumorgenesis and prolong overall survival rate. Future studies for identifying the molecular function of Gfi1 in CRC may improve our current understanding of tumor progression and benefit to therapeutic strategies.

A Novel Mediator of Chronic Pancreatitis

Ka Liu[1], Yanna Cao[1,2], George H. Greeley, Jr[2] and Tien C. Ko[1,2]

[1]Deparment of Surgery, UTHSC-H; [2]UTMB, TX

Background: Our laboratory has shown that pancreatic levels of Gremlin, an endogenous bone morphogenetic protein (BMP) antagonist, are elevated in both human and mouse chronic pancreatitis (CP). Deletion of Gremlin in mice attenuates CP-induced fibrosis. These results suggest that Gremlin is pro-fibrogenic in CP. However, the underlying mechanism is unknown. We hypothesize that Gremlin blocks the anti-fibrogenic BMP signaling, leading to pancreatic fibrosis in CP.

Objective: Determine the role of BMP signaling in Gremlin-induced pancreatic fibrosis.

Methods: PSCs were isolated from wild-type (wt) and BMP receptor type II knockout mice ($BMPR2^{+/-}$). The cultured PSCs were treated with vehicle, Gremlin (500 ng/ml), BMP2 (50 ng/ml), TGF-β1 (1 ng/ml), or various combined treatments. Activation of BMP signaling was assessed by measuring phospho(p)Smad1/5 level using Western blotting. Fibronectin (FN), an extracellular matrix protein, was measured by immunofluorescence as a functional outcome of fibrosis.

Results: In wt PSCs, compared to vehicle, BMP2 induced pSmad1/5, which was inhibited by Gremlin (12.5- vs 2.9-fold with the treatment of BMP2 vs Gremlin+BMP2, $p<0.05$). TGF-β induced FN, BMP2 inhibited TGF-β's effect, and Gremlin blocked BMP2's effect (4.7-, 1.2-, 4.8-fold with the treatment of TGF-β, BMP2+TGF-β, Gremlin+BMP2+TGF-β respectively, $p<0.05$). Furthermore, Gremlin alone induced FN by 2.3-fold in wt PSCs and this effect was attenuated by 78% in $BMPR2^{+/-}$ PSCs ($p<0.05$).

Conclusions: Gremlin promotes pancreatic fibrosis through its blockade of BMP signaling and BMPR2 is required for Gremlin's pro-fibrogenic function. Together, we have identified Gremlin as a novel mediator of pancreatic fibrosis in CP. Strategies to target pancreatic Gremlin may release repression of BMP signaling, leading to an innovative CP therapy.

Stool microbiome composition and predicted function reflect differential arginine metabolism in a multi-national cohort of women

J.L. Cope (1,2), J.W. Hsu (3), P. Dwarkanath (4), J. M. Karnes (3), C.C. Kao (3), R.A. Luna (1,2), M.M. Thame (5), A.V. Kurpad (4), J. Versalovic (1), E.B. Hollister (1,2), F. Jahoor (3)
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Despite adequate dietary intakes of energy and protein, Indian women give birth to low birth weight (LBW) babies at high rates. Previous research has shown that, although women from other nations increase arginine production during pregnancy, Indian women do not, and there is a strong association between the prevalence of LBW babies and reduced arginine production. In these cases, reduced bioavailability of arginine was not due to inadequate dietary intake but rather to increased catabolism by arginase, possibly by the gut microbiota.
To investigate whether the gut communities of Indian women differ in composition and arginine utilization and metabolism from American and Jamaican we recruited a multi-national cohort (n=27). Their stool microbial communities, dietary habits, and metabolic characteristics were evaluated, including total carbohydrate and energy intakes, gut absorptive capacity, and the kinetics of several arginine metabolic pathways. 454 pyrosequencing was used to sequence the V3V5 region of the 16S rRNA gene from stool. Sequences were assigned to operational taxonomic units (OTUs), and the OTU assignments were used to generate functional predictions, which were compared among subjects.

Three dominant community types were identified among the stool specimens and were characterized by increased abundances of genera *Prevotella*, *Bacteroides*, or *Bacteroides* with *Clostridium*, respectively. These dominant states tended to reflect the geographic origin of their samples, but not exclusively so. Subjects harboring *Prevotella*-dominant communities had lower gut absorptive capacity and a lower endogenous arginine flux, while communities containing higher abundances of *Clostridium* showed a marked increase in both gut absorptive capacity and endogenous arginine flux. Subjects with greater body weight and gut absorptive capacity were more likely to have a *Bacteroides*-dominated community even with a converse low energy intake and low total carbohydrate intake possibly due to high gut absorptive capacity values. Prevotella-dominant or the mixed *Bacteroides* with *Clostridium*-dominant type occurred in subjects with lower body weight, higher energy and total carbohydrate intake, and lower gut absorptive capacity. Congruent with physiological measurements of arginine flux and the three dominant fecal community types detected among the members of this multi-national cohort, predictions of metagenomic function suggest that the conversion of arginine to ornithine is likely to be higher in the *Bacteroides*-dominant communities. Likewise, the further conversion of ornithine to citruline is likely to be lower in the *Prevotella*-dominated communities as they largely lack the *Firmicute* genera *Lachnospiraceae* and *Ruminococcaceae*.

We demonstrate that the gut microbiota vary in conjunction with arginine metabolism and have the potential to impact arginine availability in the human host. Modulation of the gut microbiota may represent a future treatment for inadequate arginine availability during pregnancy and decrease the risk for LBW babies.

Impaired rotavirus viroplasm formation and maturation in protein kinase-inhibited cells provides new clues into cellular lipid droplet formation

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Rotavirus (RV) replication occurs in cytoplasmic inclusions, called viroplasms, whose formation requires the interaction of RV nonstructural proteins NSP2 and NSP5 and components of lipid droplets (LDs). RV infection induces LD formation and nascent viroplasms form on LDs at ~2-3 hours post infection (hpi). LD/Viroplasms increase in size into larger, mature viroplasms by ~5-6 hpi. Mature LD/viroplasms enclose the viral genome replication machinery and are surrounded by intracellular membranes containing RV nonstructural protein NSP4. How RV induces LD formation is unknown.

We recently reported that two forms of NSP2 are produced during RV infection. A cytoplasmically dispersed form of NSP2 (dNSP2) appears at ~2 hpi, and a viroplasm-associated form (vNSP2) appears at ~3 hpi. Each form of NSP2 interacts differentially with specific phospho-isoforms of NSP5: dNSP2 only associates with *hypo*-phosphorylated NSP5 while vNSP2 associates exclusively with *hyper*-phosphorylated NSP5, suggesting a phosphorylation-dependent mechanism for viroplasm formation. Since no RV-encoded protein kinase has yet been described, we assessed the requirement for cellular protein kinases in RV replication using target-specific inhibitors.

We evaluated the roles of casein kinase I (CKI) and casein kinase II (CKII) on viroplasm formation because each kinase has been implicated in NSP5 phosphorylation and NSP2-dependent NSP5 hyper-phosphorylation. Cells were pre-treated with inhibitors to one or both kinases (or vehicle) prior to infection and assayed by immunofluorescence microscopy (IF) and western blot analysis. Infectious yield was determined by fluorescent focus assay. IF demonstrated that viroplasms in CKI/CKII-inhibited cells showed a delay in viroplasm formation, dramatically decreased viroplasm size, impaired lipid droplet association, and little or no association with NSP4. Western blot analysis demonstrated a decrease in NSP5 hyper-phosphorylation.

Our results suggest that both CKI and CKII have a role in viroplasm growth and maturation, which may lead to the discovery of a new phosphorylation-dependent mechanism of viroplasm assembly.

The microbiota conditions CX_3CR1^+ mononuclear phagocytes to limit inflammatory T cell responses in the intestine.

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The intestinal immune system must protect the host from pathogenic microorganisms while maintaining a symbiotic relationship with an enormous load of resident microorganisms (the microbiota). A growing body of evidence demonstrates that interactions between the immune system and the microbiota are essential for human health. Thus, a number of mechanisms limit pathological inflammation against the microbiota. Breakdown of these mechanisms is thought to underlie the development of inflammatory bowel diseases (IBD). As the microbiota displays the same immunostimulatory molecules as pathogens and can trigger inflammation and disease upon dysregulation, a critical question is how the intestinal immune system mounts protective responses to pathogens while avoiding inflammatory responses against the microbiota.

In order to prevent and treat IBD, we must first understand the cellular and molecular processes limiting intestinal inflammation. One critical intestinal cell population is a CX_3CR1 expressing mononuclear phagocyte (MNP). CX_3CR1^+ MNPs isolated from human colon are highly phagocytic and efficient at killing intracellular bacteria. This population expands in the colon of IBD patients as well as in mouse colitis models, but their role in the normal intestine as well as in IBD is unclear, with evidence of both pro- and anti-inflammatory behavior. We recently demonstrated two ways CX_3CR1^+ MNPs integrate signals from the microbiota to limit intestinal inflammation and promote mucosal healing. First, recognition of the microbiota limits CX_3CR1 MNP migration from the intestine to the mesenteric lymph node. We hypothesize that this limits microbiota directed T cell responses. Second, signals from the microbiota induce CX_3CR1^+ MNPs to promote mucosal barrier function and repair both in mice and humans through the regulation of innate lymphoid cells (ILCs). We hypothesize that CX_3CR1^+ cells respond to microbiota-derived signals to maintain intestinal homeostasis by limiting inflammation and promoting barrier function, and that these pathways are dysregulated in IBD.

To study the *in vivo* role of these cells, we generated novel mouse strains, in which CX_3CR1^+ MNPs can be selectively depleted. We used these mice to understand the role CX_3CR1^+ MNPs play in modulating T cell responses to intestinal antigens. In the presence of the intact microbiota, we identify CX_3CR1^+ MNPs as critical for limiting the induction of inflammatory intestinal T cell responses. They are critical both for inducing tolerance to soluble protein antigens as well as limiting inflammatory immune responses against pathogens. We further demonstrate that antigen presentation by CX_3CR1^+ cells is critical for priming these T cell responses *in vivo*. In contrast, in conditions where the microbiota has been perturbed, CX_3CR1^+ MNPs promote the induction of inflammatory T cell responses. Together, our findings indicate that, in the presence of the intact microbiota, CX_3CR1^+ cells are critical for intestinal homeostasis through promotion of anti-inflammatory T cell responses. Disturbances in the microbiota, such as those that occur during IBD may promote inflammatory behavior of these cells leading to a loss of intestinal homeostasis. Understanding this regulation of CX_3CR1^+ MNPs will open up new therapeutic targets for limiting intestinal inflammation and the treatment of IBD.

The role of the gut microbiome in obstructive sleep apnea induced hypertension

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Obstructive sleep apnea (OSA), characterized by repeated closure of the upper airway during sleep, is a significant clinical problem affecting upwards of 25% of the adult population in the United States. OSA has been shown to be an independent risk factor for systemic hypertension, and the most common underlying cause of resistant hypertension. While the etiology of OSA -induced hypertension is likely multifactorial, inflammation is considered an integral component in the pathological cascade. The source of the inflammation associated with OSA is not presently known. **We hypothesized that alterations to the gut microbiome during OSA produces low grade inflammation and ultimately hypertension.** Airway obstructions, simulating apneas, were induced by chronically implanting an inflatable obstruction device (OD) in the trachea of rats. The ODs were remotely controlled by a computer to produce 60 apneas/hr (10 sec. each) during 8 hrs of the sleep cycle in free-ranging rats. We have previously demonstrated that young healthy rats (8 weeks old) on a normal chow diet do not develop hypertension following 4 weeks of OSA. Additionally, sham rats (underwent surgery without apneas) fed a high fat diet do not develop hypertension. However, when rats were fed a high fat diet for 3 weeks prior to apneas, they exhibited a significant elevation in systolic blood pressure after 1 (19mmHg; n=10-13, $p<0.05$) and 2 weeks (21mmHg; n=10-11, $p<0.05$) of apneas. Interestingly, when rats fed a high fat diet were treated with oral antibiotics (ampicillin and neomycin), resulting in a significant decrease in Firmicutes (81% to 2%, n=3-6, $p<0.05$), OSA-induced hypertension was abolished. These observations led us to examine changes in the gut microbiome following high fat diet, which may be required for OSA-induced hypertension. Fecal samples from normal chow and high fat diet rats were sequenced for the 16s rRNA gene to evaluate the microbiome diversity and composition. Compared to normal chow, high fat diet led to a significant increase in Firmicutes (49% vs. 81%; n=4-6, $p<0.05$) and decrease in Bacteroidetes (48% vs. 4%; n=4-6, $p<0.05$). To further demonstrate the importance of dysbiosis in OSA-induced hypertension we transplanted cecal contents isolated from hypertensive OSA rats on high fat diet into normotensive OSA rats that had been previously treated with oral antibiotic. Recipient rats developed hypertension within 7 days of OSA (n=4, $p<0.05$). In conclusion, these data demonstrate a key role for the gut microbiome in the development of OSA-induced hypertension.

Human gut microbiota alter intestinal mucus in HT29-MTX-E12 cells

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Intestinal mucus, consisting of glycosylated mucin proteins, provides a protective barrier for the host and a matrix for bacterial adhesion/colonization under normal homeostatic conditions. Alterations in mucin expression and/or mucin glycosylation are associated with the development of cancer. The aberrant expression of mucins with distinct oligosaccharide structures protects tumors from adverse growth conditions during invasion and metastasis. Although tumor-associated mucin changes are well documented, little is known about the interaction of the gut microbiota with cancer-associated mucins. To address this gap in knowledge, the mucus producing adenocarcinoma cell line HT-29-MTX-E12 was incubated with 16 human gut microbiota species under anaerobic conditions. In contrast to healthy colon, which secretes MUC2 mucin, HT29-MTX cell secrete high levels of MUC5ac and contain increased amounts of transmembrane MUC1 and MUC3. Incubation with *Clostridium coccoides, C. butryicum, B. product, Akkermansia muciniphila, Enterococcus faecium, Lactobacillus brevis and Bifidobacterium longum* induced release of MUC5ac from goblet cells. The species C*. coccoides, Bacteroides thetaiotaomicron, A. muciniphila, L. reuteri, Ruminococcus torques, B. bifidobacterium* and *B. infantis* decreased Muc5ac gene expression, while these bacterial species did not alter Muc2 expression. Strain-specific alterations were observed for transmembrane mucins Muc1, 3 and 4. Changes in mucin gene expression were correlated with Periodic Acid Schiff- Alcian blue (PAB-AB) stains, that detect mucin proteins of HT29-MTX monolayers. Microbes adhered poorly to coated HT29-MTX mucus compared with healthy human stool mucus, indicating altered adhesion with alter oligosaccharide content. Bacterial-specific alterations of MUC5ac may yield new combination treatments for enhanced access of therapeutic agents to colonic tumors of the intestinal mucosa.

Attenuation of colonic inflammation and inflammation-associated colorectal cancer by probiotic *Lactobacillus reuteri*

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Background: Supplementation with probiotic *Lactobacillus* strains has been effective at ameliorating intestinal inflammation and inflammation-associated colonic cancer in rodent models, but the underlying mechanisms are unknown. Previous studies showed that *L. reuteri* strains harboring a complete *hdc* gene cluster which is responsible for the synthesis and secretion of histamine from L-histidine suppress TNF production in activated THP-1 cells, indicating a potential role of histamine in alleviation of inflammation.

Methods and Results: Using a trinitrobenzene sulfonic acid-induced mouse model of colitis, oral administration of *hdc*+ *L. reuteri* strain 6475 protected Balb/c mice from colitis. Suppression of colitis was indicated by decreased weight loss, amelioration of colonic injury and reduced serum amyloid A protein concentrations. Mice receiving *L. reuteri* 6475 also showed decreased expression of pro-inflammatory cytokine genes IL-6 and IL-1β, as well as reduced uptake of [18F]fluorodeoxyglucose (18F-FDG) in the colon by positron emission tomography (PET). Further studies showed that suppression of colitis depended on introduction of an intact histidine decarboxylase gene (*hdcA*) into the intestinal microbiome, consumption of a histidine-containing diet and signaling via the histamine H2 receptor. Moreover, using an azoxymethane/dextran sodium sulfate-induced inflammation-associated colorectal cancer model, oral administration of *L. reuteri* 6475 significantly decreased the number and size of colonic tumors and uptake of 18F-FDG by PET in the colon of male $Hdc^{-/-}$ mice compared to control mice that received media only. Meanwhile, the *hdcA* isogenic mutant of *L. reuteri* 6475 which lacks the histamine producing activity did not show such anti-carcinogenic effects, indicating a role of the *hdcA* gene and production of histamine in suppressing carcinogenesis.

Conclusion: These combined investigations indicate that *hdc*+ *L. reuteri* attenuates experimental colitis and inflammation-associated colonic cancer via conversion of L-histidine to histamine. These findings link luminal conversion of dietary components (amino acid metabolism) by the intestinal microbiome and probiotic-mediated suppression of colonic inflammation and cancer. The effective combination of diet, probiotic and disease phenotype may result in opportunities for therapeutic microbiology.

Glucagon-like peptide-2 reprograms glucose metabolism for proliferation in intestinal stem cells

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Background: Emerging evidence suggests that **a**) glucose metabolism (aerobic glycolysis) is reprogrammed for stem cell proliferation; and **b**) intestinal metabolic reprograming of glucose is coupled with crypt cell proliferation after Roux-en-Y gastric bypass surgery. Very little is known about which signals drive the metabolic reprogramming of glucose and cell proliferation in intestinal adaptation/regeneration. **Glucagon-like peptide-2 (GLP-2)** is an enterotrophic hormone, and approved by the FDA to treat short bowel syndrome and Crohn's disease. GLP-2 has proabsorptive, proliferative, cytoprotective, and anti-inflammatory actions in the gut. GLP-2-induced pleiotropic actions are mediated through the distinct GLP-2 receptor (GLP2R), yet its direct action and signaling in intestinal epithelium are poorly defined. GLP2R is expressed in crypt secretory precursors, while GLP-2 is secreted from villous enteroendocrine L cells. **Hypothesis and Aims:** We hypothesized that GLP2R activation in intestinal stem cells (ISC) reprograms metabolic fates of glucose to support proliferation by triggering aerobic glycolysis and activating PKM2 signaling pathway. To address if the nutrient-responsive GLP-2 is a key instructive signal for coupling ISC metabolic reprogramming and proliferation, we wanted to determine if and how GLP2R directly modulates metabolic reprogramming of glucose and cell proliferation in human/ mouse mini-guts. **Methods:** Human/ mouse jejunal crypts were isolated and cultured in Matrigel. Intracellular ^{13}C-metabolic fluxes and cell proliferation in the mouse mini-guts were quantified by U-^{13}C-d-glucose tracer and BrdU incorporation. The mini-guts were treated with GLP-2 (100 nM) ± glycolysis inhibitor (3-bromopyruvate, 10 μM) and labeled with U-^{13}C-d-glucose tracer or BrdU for 90~120 min. **Results:** We show that **a**) the GLP2R was required for the optimal growth of mouse mini-guts; and for GLP-2 to augment cell proliferation in an aerobic glycolysis-dependent manner. **b**) Using LC-MS/MS-based metabolomics, we revealed that GLP-2 shifted ^{13}C-glucose metabolic fluxes to aerobic glycolysis and *de novo* biosynthesis of glutamine in the mini-guts. This metabolic reprogramming and cell proliferation were negated by glycolysis inhibition. **c**) Using proteomics, we identified that GLP2R was engaged in glycolysis via interacting with GAPDH (a key glycolytic enzyme). GLP-2 acutely induced phosphorylation and nuclear translocation of pyruvate kinase M2 (PKM2, a key isoform mediating the Warburg effect) in the mini-guts. **Conclusions:** We conclude that GLP-2 plays a novel role in metabolic reprogramming of glucose for biosynthesis to support cell proliferation in the crypt stem cells. Thus, GLP-2 may be the dual (metabolic and mitogenic) signal for coupling metabolic reprogramming and cell proliferation in the crypt stem cells, a key fundamental aspect of intestinal adaptation/ regeneration after surgery. (This project was supported in part by NIH grant DK56338, which supports the Texas Medical Center Digestive Diseases Center.)

The cytoskeletal regulator p120 Catenin suppresses epithelial cell delamination in preinvasive pancreatic cancer

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By 2020, pancreatic cancer is estimated to climb from the 4th to the 2nd most common cause of cancer-related deaths in the United States. This deadly disease has continued to remain largely refractory to chemotherapeutic and treatment regimens, and patients often experience a heavy metastatic burden. A study combining the Sleeping beauty transposon random insertion mutagenesis system with an oncogenic Kras^{G12D} allele in mice as a screen to identify candidate pancreatic cancer genes identified genes encoding adherens and tight junction proteins as significantly enriched in Kras-driven neoplasia with Ctnnd1 as a locus commonly mutated in mice developing metastatic progression of pancreatic cancer. Ctnnd1 encodes the adherens junction protein p120 catenin, which is integral in stabilization of cadherin molecules at cell membranes. Misexpression of p120 catenin in primary resected pancreatic tumors correlates with worse survival in pancreatic cancer patients yet, the mechanisms by which p120 catenin contribute to the pathogenesis of pancreatic cancer are not clear. We comprehensively examined p120 catenin expression in human pancreatic intraepithelial neoplasia (PanIN) and identified mislocalization of p120 catenin to the cytoplasm as early as PanIN2. 3/5 PanIN3 examined had predominant cytoplasmic staining, which led us to hypothesize that p120 catenin may play a critical role in early pancreatic neoplasia, before the onset of pancreatic ductal adenocarcinoma. To examine the role of p120 catenin during early Kras-driven pancreatic neoplasia, we ablated p120 catenin in a mouse model of preinvasive pancreatic cancer, KC^{iMist1}. KC^{iMist1}p120$^{f/f}$ pancreases display significant acceleration of acinar to ductal metaplasia (ADM) and PanIN formation when compared to KC^{iMist1}p120$^{wt/wt}$ pancreases one month post tamoxifen injection. At the same time point, KC^{iMist1}p120$^{f/f}$ pancreases are significantly larger than KC^{iMist1}p120$^{wt/wt}$ pancreases with 92.89% pancreatic area occupied by fibrostroma. As a result, KC^{iMist1}p120$^{f/f}$ animals have severe exocrine pancreatic insufficiency and die on average 8 weeks earlier than their KC^{iMist1}p120$^{wt/wt}$ controls. Lineage tracing revealed remarkable epithelial cell delamination in KC^{iMist1}p120$^{f/f}$ pancreases. Quantification of isolated epithelial cells in the extensive stroma revealed a striking 832/7000 CK19^{+} cells in KC^{iMist1}p120$^{f/f}$ pancreases vs. 15/7000 CK19^{+} cells in KC^{iMist1}p120$^{wt/wt}$ pancreases. Microarray analysis showed >1263 differentially expressed genes! IPA pathway analysis revealed significant differential gene expression in PI3K/AKT and Cdc42 signaling. Taken together, our results suggest a critical role for p120 catenin in regulating epithelial cell delamination in early pancreatic cancer.

Unbiased Proteomic Analysis of Esophageal Eosinophilia Reveals Differential Expression of Galectin-3

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Introduction: Esophagus is typically devoid of eosinophils. Persistent esophageal eosinophilia (EE) has been described in association with eosinophilic esophagitis (EoE) - an allergen-mediated, clinicopathological condition characterized by eosinophil driven inflammation limited to the esophagus which can eventually result in epithelial-mesenchymal transition and narrowing of the esophageal lumen. Although some molecular mechanisms related to EE have been identified, the tissue protein alterations remain unclear.

Aims: To determine relative global protein abundance and redox state (cysteinyl-S-nitrosylation; SNO) in esophageal tissue obtained from children with EE compared to esophageal tissue obtained from healthy children.

Methods: Proteins were extracted and separated by 2D-gel electrophoresis (2DE) from 2-4 proximal and distal esophageal (PE and DE, respectively) biopsies obtained from 6 children with EE and 7 Controls (≤ 1 eos/hpf with no histologic evidence of inflammation). Normalized spot volumes and ratio-of-ratio (RoR) exhibiting $p \leq 0.05$ and ratios of abundance and RoRs≥1.5 were selected for robotic picking, and analyzed by matrix-assisted laser desorption ionization time-of-flight tandem mass spectrometry (MALDI TOF/TOF). The proteins abundance was analyzed by t-tests, principal component analysis, and unsupervised hierarchial clustering. Candidate proteins were confirmed by western blot of tissue samples.

Results: Both groups were comparable for age, gender, and presenting symptoms. Food allergy was noted in 67% of children with EE and the median eos/hpf was 38 (range: 17-100). None of the controls reported any food allergy. Proteomic analysis of mucosa from patients and controls revealed a total of 648 proteins, and 91 statistically significant ($p \leq 0.05$) protein spots were subjected to protein identification by MALDI MS, and the 62 exhibiting protein scores of 56 or higher were considered high confidence identifications. Eight unique proteins were significantly upregulated and 3 proteins were down-regulated in PE. DE contained more differentially abundant proteins where 6 were upregulated and 8 were down regulated. Expression of galectin-3 (Gal-3), a galactose-specific lectin involved in acute allergic inflammatory responses, was uniquely elevated >1.6 fold in PE biopsies and >2.1 fold in DE biopsies.

Conclusion: Our unbiased proteomic analysis of EE and normal esophageal reveals a distinct protein abundance signature. With western blot confirmation, Gal-3 appears to be associated with disease phenotype and may be a major contributor to eosinophilic esophageal inflammation and related esophageal remodelling. Additional studies to confirm these findings are underway.

Gut dysfunction permits the transmission of genetic Information between plants and consumers

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The prevailing scientific view suggests the consumption of plant-based dietary RNAs has no influence on the healthy; however, our data suggest that consumers with diet or substance-related alterations in intestinal permeability may uptake and circulate these RNAs. After several days on a honeysuckle (*Lonicera japonica*) containing diet, gavage fed microRNAs (miRNAs) could be detected in the sera and urine of animals. In the honeysuckle fed population, plant based small RNAs could also be detected without gavage feeding. In the honeysuckle fed animals, the dietary small RNAs appear in circulation after several days on the diet and were no longer detectable 48 hours after the honeysuckle was removed from the diet. RT-PCR demonstrated dose dependent uptake of gavage fed exogenous RNAs and digital droplet PCR showed that the amplified RNAs were of dietary origin. Cisplatin, a chemotherapy drug, was used to induce gut/kidney damage in mice and gavage-fed exogenous miRNAs could be detected in the sera of the cisplatin-treated mice without dietary manipulations. Microscopic investigation and FITC-Dextran assays demonstrated altered permeability and gut architecture in the cisplatin mice, but not in the honeysuckle fed consumers. We propose a model where an array of diets and disease states may influence the incidence of exposure to dietary small RNAs. Establishing conditions whereby diet-derived nucleic acids are absorbed and functioning in the consumer will drastically alter our concept of the relationship between health and nutrition, help establish useful dietary practices, and potentially open up new vistas for gene therapy.

Norwalk virus shedding duration is associated with stability of the fecal microbiome

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The gut microbiome contributes to the development of gut immunity and enhances enteric viral replication and systemic pathogenesis. We assessed the interaction between norovirus, the intestinal microbiota, and the human host in fecal samples collected from the Norwalk virus challenge study. The study population consisted of 46 genetically susceptible individuals who participated in an experimental challenge with Norwalk virus, the prototypical norovirus strain. Fecal samples collected at days -7, 2, 4, 8, 21, and 56 were analyzed to assess changes in the fecal microbiota related to Norwalk virus infection. The pre-challenge fecal microbiome composition was not associated with susceptibility to NV infection, and microbiome composition post-challenge was not associated with the presence of gastroenteritis or specific symptoms. At pre-challenge and 56 days post-challenge, the microbiome composition of infected and uninfected individuals was similar. Among the 20 infected individuals, the microbiome of long shedders (≥ 18 days viral shedding) was more stable when compared to pre-challenge than that of short shedders (≤ 14 days viral shedding). Furthermore, metagenomic sequencing revealed increased abundance of *Subdoligranulum variabile*, a butyrate producer, and butyrate metabolism pathway genes in long shedders. The results indicate that Norwalk virus infection is not associated with fecal microbiome composition. However, microbiome stability is associated with viral shedding duration. We demonstrated that long shedders maintain a stable microbiome characterized by increased potential for butyrate production throughout Norwalk virus infection. Further characterization of the relationship between the microbiome and intestinal health may improve understanding of norovirus replication in the human host.

Store-operated and voltage-activated calcium channels are activated by rotavirus NSP4-mediated viroporin activity through release of ER calcium stores

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Rotavirus (RV) is the leading cause of viral childhood gastroenteritis and, despite implementation of two licensed vaccines, causes >450,000 deaths worldwide. Our studies use a combination of *in vitro*, *in vivo*, and newly developed human intestinal enteroid model systems to understand the molecular mechanisms of RV-induced changes in Ca^{2+} signaling that contribute to diarrheal disease. A hallmark of RV infection is elevated cytoplasmic Ca^{2+} ($[Ca^{2+}]c$) caused by release of endoplasmic reticulum (ER) Ca^{2+} stores and Ca^{2+} entry through plasma membrane (PM) Ca^{2+} channels, all of which is essential for RV replication and thought to contribute to secretory diarrhea. We showed RV nonstructural protein 4 (NSP4) is a viroporin (viral pore-forming protein) and this function elevates $[Ca^{2+}]c$, but the molecular mechanisms of how NSP4 viroporin activity disrupts host Ca^{2+} homeostasis and how these signals are integrated to chloride secretion and diarrhea remained uncharacterized. We hypothesized that NSP4 forms a Ca^{2+} channel to release luminal ER Ca^{2+} and ER Ca^{2+} depletion activates store-operated (SOCE) and voltage-activated (VACC) Ca^{2+} channels in the PM. Ca^{2+} entry through these channels subsequently activates Ca^{2+}-activated chloride channels (CaCCs) to induce Cl^- secretion that causes diarrhea. We have generated a series of stable cell lines that express fluorescent Ca^{2+} or Cl^- biosensors. To evaluate effects of NSP4 on SOCE, we generated a biosensor cell line stably expressing YFP-tagged stromal interaction molecule 1 (YFP-STIM1), an ER transmembrane sensor of luminal Ca^{2+} that activates SOCE upon ER Ca^{2+} store release. We found that RV infection or recombinant NSP4 expression activated YFP-STIM1 and elevated $[Ca^{2+}]c$, but a NSP4 viroporin mutant failed to activate YFP-STIM1 and did not increase $[Ca^{2+}]c$. Using both human embryonic kidney (HEK293FT) and human intestinal (Caco2BBe) cell lines stably expressing a GFP-based Ca^{2+} sensor, we found that RV induced Ca^{2+} entry was inhibited by multiple blockers of the SOCE Orai1 Ca^{2+} channel, most potently by the drug 2-APB. Preliminary results using a halide-quenched YFP-biosensor cell line to measure Cl^- secretion, indicate the Anoctamin 1 (Ano1) CaCC is strongly activated by RV and blocking Ca^{2+} entry through Orai1 with 2-APB inhibited CaCC activation, suggesting SOCE-mediated Ca^{2+} entry is critical for Cl^- secretion, which is likely responsible for diarrhea. SOCE Ca^{2+} influx inhibitors all reduced RV yield, underlining the importance of this process for RV replication. Unexpectedly, L-type voltage-activated Ca^{2+} channel (VACC) blockers also significantly reduced RV replication and we discovered that human enterocyte cell lines and human intestinal enteroids all express the $Ca_v1.3$ VACC. Specific VACC blockers inhibited both NSP4-induced Ca^{2+} entry and RV replication, suggesting $Ca_v1.3$ is important for RV-induced Ca^{2+} uptake. Our studies show that RV NSP4 releases ER Ca^{2+} to activate STIM1, triggering Ca^{2+} entry through host Ca^{2+} channels in the PM, including SOCE and VACC channels. This global disruption of Ca^{2+} homeostasis directly activates CaCC chloride secretion and supports RV replication. It is possible other enteric viruses (e.g., noroviruses) disrupt host Ca^{2+} signaling, making these pathways a general mechanism for viral gastroenteritis. If so, drugs targeting cellular Ca^{2+} channels, such as Orai1 and $Ca_v1.3$, should be studied as potential antiviral drugs to reduce gastrointestinal virus-induced diarrheal disease.

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Special considerations for fecal microbiota transplantation in pediatric recurrent *Clostridium difficile* infection

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BACKGROUND: Fecal microbiota transplantation (FMT) is the most effective treatment for recurrent (antibiotic refractory) *Clostridium difficile* infection (rCDI). Special considerations for FMT must be taken with pediatric rCDI, given a higher asymptomatic carriage rate and more frequent underlying clinical conditions when compared to adults. We performed metagenomic analyses to evaluate the microbial mechanism of action of FMT in a cohort of rCDI patients, with the incorporation of these pediatric considerations.

METHODS: Nine rCDI patients (1.5-16 year old) received filtered, frozen-thawed fecal preparation from screened, self-designated or universal donors through colonoscopy under an IRB approved protocol, followed by enema FMT, if clinically indicated. Two patients had inflammatory bowel disease (1 ulcerative colitis [UC], 1 Crohn disease [CD]), 1 had heart transplant, and 2 had significant neurologic impairment as underlying conditions. All patients provided a stool sample 1 day prior and 8 weeks after the first FMT. Select patients gave additional samples to be analyzed. The fecal microbiomes were studied by 454 pyrosequencing of the bacterial 16S rRNA gene.

RESULTS: All 4 patients without underlying disease had resolution of their symptoms for more than 2 months following a single FMT. One of these patients was found to be positive for *C. difficile* 4 months after FMT during an upper respiratory infection, but was without gastrointestinal complaints. He was considered an asymptomatic carrier. Metagenomic analyses indicated that FMT induced convergence of the recipient microbiomes to the donor's microbiome. Recipient microbiomes, however, remained as distant from the donor microbiome as independent healthy children and adults. *Bacteroidetes* specifically increased in abundance following FMT. Interestingly, asymptomatic carriage of *C. difficile* did not significantly modify microbiome composition.

Out of the 5 patients with complicating clinical conditions, only 2 had obvious clinical benefit from *C. difficile* directed antibiotic therapies prior to FMT. These patients responded well to a single FMT. The UC patient required FMT 3 times to clear CDI, but still received colectomy during his subsequent clinical course. The CD patient's diarrhea did not respond to either antibiotics or FMT. One patient was diagnosed with poorly managed constipation and *C. difficile* carriage during her 2nd enema FMT.

CONCLUSIONS: FMT is highly effective for the treatment of pediatric rCDI in patients without complicating clinical conditions. Clinical improvement during *C. difficile* directed antibiotic therapy in the course of rCDI indicates a favorable outcome following FMT. FMT results in engraftment of donor bacterial species and reconstitution of recipient microbiomes. Asymptomatic *C. difficile* carriage may predispose to CDI. A special approach incorporating these findings should be taken when considering FMT for rCDI in pediatric patients with underlying disorders.

Characteristics of mucosal and fecal microbiota in healthy individuals

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Background: Our understanding on longitudinal spatial diversity of mucosal microbiota in the large intestine, as well as on the diversity at lower taxonomic level between mucosal and fecal microbiota in healthy individuals is limited.

Methods: We enrolled eight men (age: 51-69 years old, four non-Hispanic white) from the colonoscopy suite at the Michael E. DeBakey VA Medical Center in Houston between July 2013 and July 2014. We obtained a total of 38 snap frozen normal colonic mucosa surface biopsies from cecum, ascending, transverse, descending, sigmoid, and rectum segment of the intestine. The fecal samples were also collected from three men before bowel cleaning. Microbial DNA was extracted from samples and the 16S V4 region was amplified and sequenced using the Illumina MiSeq platform. The sequencing data were analyzed using QIIME v1.8 for operational taxonomy unit (OTU) classification and calculation of diversity indices.

Results: We obtained ~3,450 sequences for each sample. Feces and colonic mucosa had comparable amounts of OTUs identified for each subject. Bacteroidetes, Firmicutes, and Proteobacteria accounted for > 95% of both mucosal and fecal samples. Nonetheless, feces had increase abundance of Proteobacteria compared to colonic mucosa, In contrast, colonic mucosal samples showed an increase in Firmicutes and Bacteroidetes compared to fecal samples. Each subject had unique microbiota signature. Variations in composition were seen for Lachnospiraceae, Escherichia_Shigella, Akkermansia, and Faecalilbacterium at the Genus level. For each subject, the composition of the mucosal microbiota along the large intestine remained stable at the phylum level. Variations in compositions were seen for Akkermansia and Lachnospiraceae at the genera level. The Shannon diversity index scores were higher for colonic mucosa than for feces suggesting a more equal distribution in the abundances of the OTUs found in mucosal sample. The beta-diversity analysis (weighted UniFrac PCoA) suggested that the microbiome was clustered by each individual rather than by sample segment. For the same individual, the separation was seen for rectum from other segment.

Conclusions: The intra-individual microbiota diversity was greater than the inter-segment diversity among each individual. Mucosal microbiota was under-presented in the fecal samples. The implication of mucosal and fecal microbiota in colon tumorigenesis may be different. Host and environmental determinants of longitudinal variation of microbial community should be investigated.

Effects of *L. reuteri* derived histamine on IL-8 expression using *in vitro* cell culture models

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Probiotics may affect the disease course of patients with chronic immune-mediated disorders such as inflammatory bowel disease via modulation of the host immune system. One such microorganism, *Lactobacillus reuteri* ATCC PTA 6475, has been shown to convert a dietary component, L-histidine, to the biologically active immunoregulatory signal histamine via a histidine decarboxylase. The produced histamine from *L. reuteri* has been shown to suppress the pro-inflammatory cytokine TNF in human myeloid cells thereby modulating host immunity. However, as the primary contact of the *L. reuteri* derived histamine is the intestinal epithelial cell (IEC) layer, our project sought to identify how histamine affects the expression of cytokines from epithelial cells. Our studies showed that *L. reuteri* derived histamine significantly suppresses the expression of the pro-inflammatory cytokine IL-8 by HT-29 cells stimulated with IL-1β, compared to that of the control groups without histamine. In addition, HT-29 cells treated with commercially available histamine showed decreased expression of IL-8. Overall, *L.reuteri* derived histamine shows beneficial effects by modulating pro-inflammatory cytokines

Systemically-altered lymphatic function in mice with dextran-sulfate sodium-induced acute colitis

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Background: Altered lymphatic function and structure are implicated in a number of pathological conditions including inflammation. Recent data suggests that the lymphatic system may play an important role in the pathogenesis of inflammatory bowel disease (IBD) and extraintestinal manifestations. However, it is unknown if, and how local inflammation in the gut affects systemic lymphatic function. The aim of our study was to investigate whether lymphatic function and architecture are systemically altered in dextran sulfate sodium (DSS)-induced acute colitis.

Methods: In the acute DSS colitis model, Balb/c and C57BL/6 mice and mice lacking inducible nitric oxide synthase (iNOS-/-) were fed with DSS in the drinking water and monitored to assess disease activity. Mice were also treated with both DSS and a NOS inhibitor, N^G-nitro-l-arginine methyl ester (L-NAME). Mesenteric lymphatic vessels were visualized after oral gavage of Bodipy-FL-C16. Lymphatic contractile function and vessel remodeling were longitudinally characterized using dynamic near-infrared fluorescence (NIRF) imaging following intradermal (i.d.) injection of indocyanine green (ICG) to the hind paw in mice prior to and 4 and 7 days after DSS treatment.

Results: Dynamic NIRF imaging data demonstrated systemically impaired lymphatic contractility in the skin of mice exposed to DSS. Mice with acute colitis showed impaired lymphatic drainage in the mesenteric lymphatic vessels, but not in dermal lymphatics. However, we observed dilated lymphatic vessels in the skin of mice with DSS-induced colitis. The lymphatic vessels in iNOS-/- mice exposed to DSS for 4 days showed similar lymphatic contraction frequency as baseline, but significantly higher frequency than wild-type mice with the same treatment. However, at 7 days of DSS treatment, the contractile frequency in both iNOS-/- and wild-type mice was significantly reduced as compared to their respective baselines and did not statistically differ from each other. DSS-colitis mice treated with L-NAME showed partial but not complete improvement of systemic lymphatic pump activity. Additionally, histology and fluorescence activated cell sorting analysis of peripheral lymph nodes of mice with DSS-induced colitis reveal altered architecture, leukocyte profile and dendritic cell migration.

Conclusions: Our study demonstrates shows the feasibility of functional NIRF imaging to non-invasively monitor lymphatic function and vessel remodeling in systemic lymphatic response to inflammation and provide information about response to therapy.

Cerebral Small Vessel Disease, Obstructive Sleep Apnea, and Gut Dysbiosis

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Individuals suffering from obstructive sleep apnea (OSA), a condition where the upper airway repeatedly collapses during sleep to produce apnea, are at risk for either developing cerebrovascular diseases or accelerating the progression. One of these cerebrovascular diseases, cerebral small vessel disease (CSVD), involves pathological alterations to the small vessels of the brain. CSVD produces cognitive impairment, resulting from damage to cerebral white and deep grey matter. In the present study we tested the hypothesis that CSVD is accelerated by pathological alterations to the gut microbiome (i.e., dysbiosis) resulting from OSA. 15 to 20 week old spontaneously hypertensive stroke prone rats (SHRSP), an animal model for CSVD, were chronically instrumented with a tracheal balloon that could be remotely inflated. Rats were randomly assigned to an OSA group, where the balloon was inflated 60 times/hr for 10 sec to produce apneas, corresponding to severe OSA in humans, or to a sham control group that were instrumented with a tracheal balloon but were not subjected to apneas. In the OSA group, apneas continued each day for 8 hr during the sleep cycle for 2 weeks. Analysis of the gut microbiome, through sequencing of the bacterial 16s rRNA gene in fecal samples, revealed a significant shift in the Firmicutes:Bacteriodetes ratio (from 0.76 to 0.48, $p=0.046$). Systolic blood pressure was significantly increased ($n=7$-10, $p<0.05$) in SHRSP with OSA by 20 ± 4 mm Hg above the sham rats respectively. Blood brain barrier permeability, as assessed by IgG extravasation, was significantly increased ($n=6$, $p<0.05$) in small vessels of the OSA groups compared to sham controls. Activation of microglia, the resident immune cells in brain was significantly greater (as determined by morphometric analysis) in the OSA group compared to sham controls ($n=6$, $p<0.05$). When pretreating the SHRSP OSA group with antibiotics (ampicillin [1 gm/L] and neomycin [0.5 gm/L] in drinking water), which dramatically decreased Firmicutes:Bacteriodetes ratio in another strain of rats, OSA induced no significant change in the systolic blood pressure ($n=4$-9, $p=0.478$), reduced damage to the blood brain barrier ($n=4$-9, $p=0.015$), and attenuated or abolished activation of microglia ($n=4$-9, $p=0.001$). Our studies link gut dysbiosis, occurring as a result of OSA, to hypertension, blood-brain barrier integrity, and activation of resident immunity in the brain. Our study further suggests that bacteriotherapy could possibly blunt some of the pathological consequences of OSA.

SPDEF inhibits colorectal cancer cell growth by regulating β-catenin transcriptional activity through protein-protein Interaction

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Background: Colorectal carcinogenesis is driven by a series of genetic and epigenetic changes that results in the oncogenic transformation of normal colonic mucosa. Under physiological conditions, Wingless/Int (Wnt) and Notch signaling pathways coordinately regulate homeostasis and differentiation of intestinal epithelium. However, in intestinal cancers, these pathways are frequently dysregulated. Although constitutive activation of canonical Wnt signaling resulting in high β-catenin transcriptional activity is believed to drive most human colorectal cancers (CRCs), current molecular targeted therapies have limited efficacy. Therefore, identifying transcriptional nodes between Notch and Wnt/β-catenin pathways in CRCs may allow development of new therapeutic targets and strategies. We have shown that SAM Pointed Domain Ets transcription Factor (SPDEF) is regulated by ATOH1, a key gatekeeper for Notch-driven differentiation of intestinal epithelium. SPDEF plays an important role in cell cycle exit and regulates terminal differentiation in the intestine. We have also previously reported that SPDEF is a colonic tumor suppressor that negatively regulates β-catenin transcriptional activity. Here we test the molecular mechanism of SPDEF mediated repression of β-catenin transcriptional activity in human colon cancer cells.

Material and Methods: To investigate the mechanism of SPDEF-mediated inhibition of β-catenin transcriptional activity, tagged wildtype SPDEF or truncated SPDEF mutants were transiently overexpressed in human CRC cell lines followed by β-catenin transcriptional activity assay, co-immunoprecipitation (co-IP), chromatin immunoprecipitation (ChIP) and immunofluorescent staining. We directly analyzed the effects of SPDEF expression in β-catenin-driven intestinal tumors *in vivo* by using inducible mouse model (*Lgr5*^{CreERT2}; *β-catenin*^{exon3}; *Rosa26*$^{rtta\text{-}ires\text{-}EGFP}$; *TRE-Spdef*).

Results: We found that cellular proliferation, β-catenin transcriptional activity, and expression of β-catenin targets, such as Cyclin D1 and c-Myc, is inhibited in human CRC cell lines after SPDEF expression, which is consistent with our previous studies *in vivo*. Moreover, SPDEF inhibits β-catenin-driven intestinal tumorigenesis and shrinks established tumors *in vivo*. In addition, SPDEF interacts with β-catenin in both human CRC cells *in vitro* and mouse intestinal crypts under physiological conditions. The binding region for SPDEF and β-catenin interaction was mapped. Interestingly, our results indicated that the DNA-binding domain of SPDEF is unnecessary for it to bind to β-catenin or to inhibit β-catenin transcriptional activity. Finally, our ChIP data suggested that SPDEF binds to β-catenin target sites on chromatin, resulting in the disruption of β-catenin and chromatin interaction. We propose that SPDEF could mediate other transcription programming through protein-protein interaction, independent of its DNA binding capacity, similar to what we observed for β-catenin in this study. Ongoing experiments using ChIP-seq and RNA-seq assays will reveal mechanistic insights into SPDEF function in CRC and normal intestinal biology. Taken together, these results suggest that SPDEF may be a pivotal link between Notch and Wnt/β-catenin pathways, coordinately maintaining intestinal homeostasis and impacting not only on colorectal tumorigenesis, but also intestinal stem cell biology.

Animal Model of Irinotecan-induced Fatty Liver Disease

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Fatty liver disease (FLD) is one of the leading causes of chronic liver disease in the US. Third National Health and Nutritional Examination Survey reports, in USA over 30 million adults have FLD (3 to 23 per cent). FLD manifests as simple steatosis (fat accumulation in liver), which can progress to steatohepatitis when accompanied with inflammation or fibrosis. Recent clinical studies have demonstrated that chemotherapy treatment is associated with FLD, which increases the risk of morbidity and mortality in these patients. Of particular importance is the chemotherapy drug, irinotecan which is widely used for the treatment of colorectal liver metastasis (CLM). Irinotecan based neoadjuvant chemotherapy effectively down-sizes liver metastases and thereby enables treatment of CLM by hepatic resection. However major problem with irinotecan treatment was associated FLD and morbidity/mortality after hepatic surgery. The mechanism of FLD induced by irinotecan therapy is still not known and need to be explored.

In hepatic steatosis, fat accumulation occurs by modifying free fatty acid (FFA) homeostasis including increasing hepatic FFA uptake, synthase and reducing oxidation. The mechanism involved with drugs that induce FLD seems to be diverse and unique. In certain cases, higher risk of liver toxicity has been related to enhanced generation of toxic metabolites due to increased activity of several drug metabolizing enzymes (DMEs). Irinotecan is a prodrug and its biotransformation involves (i) Bioactivation by carboxylesterases (CEs) to form more active metabolite, SN-38 which is further detoxified to inactive SN-38 glucuronide (SN-38G) by uridine diphosphate glucuronosyltransfereses (UGTs); (ii) Oxidation by cytochrome P450 (CYP) 3A enzymes to form two inactive metabolites i.e. NPC and APC. NPC is converted to SN-38 by the CEs enzymes. Excess accumulation of the SN-38 causes fatal diarrhea in cancer patients. We hypothesize that irinotecan causes FLD by altering the gene expression of enzymes involved in regulation of lipid homeostasis. Therefore, our aim is to develop irinotecan-induced FLD animal model without the bias caused by pre-existing inflammation to determine the pathogenesis and role of DMEs in this complex disease.

To develop an irinotecan-induced FLD animal model, we treated adult male C57BL6J mice with 25 & 50 mg/kg of irinotecan via intra peritoneal (i.p) route for 9 consecutive days, once daily. On day 10, liver was excised and fixed in 10% formalin for fat accumulation assessment by H & E staining. Significant steatosis (Average steatosis score: 2+) was observed at both doses of irinotecan. Interestingly, we found that irinotecan significantly down-regulated gene expression of enzyme and transcription factor involved in fatty acid synthesis, whereas up-regulated the enzyme and transcription factor involved in fatty acid metabolism. Therefore, it's reasonable to consider that most likely increased fatty acid uptake and/or decreased fatty acid output mechanisms leads to the direct accumulation of triglycerides. Importantly, the expression of Cyp3a11 was down-regulated directing possible accumulation of toxic SN38 in the liver that might contribute to the changes in fatty acid homeostasis. Thus, our novel *in vivo* model provides valuable information about the possible pathogenic mechanisms involved in irinotecan induced fatty liver. Our study is indicative of the powerful early stages of disease models that may provide important new insights in the role of DMEs in pathogenic mechanisms of complex diseases.

Dysregulation of purinergic signaling is associated with poor recurrence-free survival in patients with hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is the 2nd most lethal cancer worldwide. Recent studies suggest that extracellular ATP-mediated activation of P2 purinergic receptors induce hepatocyte proliferation *in vitro* and P2 purinergic receptors are overexpressed in certain cancer tissues. However, the pathophysiologic relevance of purinergic signaling in HCC remains unknown. **Hypothesis:** *Dysregulation of purinergic signaling facilitates aberrant cell proliferation underlying hepatocellular carcinogenesis.*

Methods: P2 purinergic receptor expression profiles (15 isoforms) of HCC patient livers (tumor vs uninvoled areas, n=42), Mst1/2$^{-/-}$ livers (hippo-deficient mice HCC) and HCC cell lines (Huh7, Hep3B, SNU387, PLC/PRF/5) were analyzed by qRT-PCR and immunohistochemistry. Findings were validated by microarray (mRNA) analysis of a larger cohort of HCC patients (n=188). Impact of purinergic signaling on HCC cell cycle progression was analyzed by Western blotting, qRT-PCR and BrdU incorporation of HCC cells exposed to ATPγS, ATP or ADP +/- SP600125 (c-Jun N-terminal Kinase inhibitor) or AF-353 (P2X3 antagonist).

Results: Multiple P2 purinergic receptor isoforms were elevated ≥2-fold in patient liver tumors compared to uninvolved areas in up to 60% of patients. High P2X3 or low P2Y13 receptor expression was associated with poor recurrence-free survival. Dysregulation of P2 purinergic receptor expression was observed in HCC cell lines. Extracellular nucleotide treatment alone was sufficient to induce cell cycle progression in Huh7 cells, as evidenced by increased BrdU incorporation and increased cyclin D3, E, and A mRNA and protein expression. JNK inhibition attenuated nucleotide-induced cyclin protein expression. Extracellular ATP-mediated activation of P2X3 promoted proliferation in HCC cells. Interestingly, ATPγS treatment induced a distinct downregulation of cyclin D1 expression in Huh7 cells, which was associated with poor prognosis in HCC patients. Mst1/2$^{-/-}$ mouse tumors exhibit dysregulated expression of multiple P2 purinergic receptor isoforms as compared to WT while nucleotide treatment of Huh7 cells induced target genes of hippo signaling, implicated in HCC pathogenesis.

Conclusions: Our analysis of HCC patients, Mst1/2$^{-/-}$ livers and HCC cells *in vitro* identifies a novel role for dysregulation of P2 purinergic signaling in the induction of a hyper-proliferative HCC phenotype and identifies P2X3 purinergic receptors as potential new targets for therapy.

An outbreak of IBD provides new evidence for a role of *M. avium paratuberculosis* in the pathogenesis of Crohn's disease

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The hypothesis that a mycobacterium, specifically *M. avium paratuberculosis* (MAP), has a role in the etiology of Crohn's Disease (CD) has been strengthened by the recent detection of MAP DNA in peripheral blood macrophages of patients with CD. A positive MAP PCR in blood specimens of up to 50% of these patients has been widely reported, but the detection rate has been variable. While PCR detection methods have improved, laboratory culture of these organisms has not advanced. A more convincing argument for MAP as a causative agent for CD would be to demonstrate a disease-specific consistency of association, compared with controls. Further, cultivation of the organism for studies of microbial genomics, molecular epidemiology, and antibiotic susceptibility would support understanding disease pathogenesis and an approach to treatment or cure with antibiotics. We hypothesized that multiple sampling of peripheral blood from CD patients would reveal a more consistent rate of detection among patients, and that with appropriate culture techniques viable organisms could be isolated. These experiments are being conducted in CD patients treated at the Houston DeBakey VA Medical Center and in an "outbreak" population of CD in a village in Bahrain, which began recently possibly after the importation of a herd of cattle afflicted with Johne's disease. The causative agent of Johne's disease is MAP. We propose that detection, isolation and culture of MAP from patients with recently diagnosed CD will demonstrate a more consistent association between MAP and CD. We will present here our observations on very high frequency of detection of MAP in peripheral blood of these two CD patient populations and early results of culture.

L-Histidine metabolism by *Lactobacillus reuteri*

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In *Lactobacillus reuteri* the decarboxylation of L-histidine to histamine by HdcA reduces the production of the pro-inflammatory molecule, TNF. We have begun to elucidate novel histidine metabolism pathways in *L. reuteri* using $^{13}C_6{}^{15}N_3$ L-histidine. Analysis of wild type and *hdcA* mutants revealed no significant changes in growth or viability when cultured in different concentrations of L-histidine or $^{13}C_6{}^{15}N_3$ L-histidine. Therefore, histamine production is not essential for *L. reuteri* growth or viability. While, there were no significant changes to growth or viability in *L. reuteri* grown in media where all histidine was replaced with carnosine (β-alanyl-L-histidine), mass spectroscopy determined that the addition of carnosine to the medium significantly increases histamine production. Together, these data have demonstrated that 1) histidine decarboxylation, although providing benefit to the host, is not required for *L. reuteri* growth; 2) the addition of carnosine improves histamine production; and 3) *L. reuteri* strains can import, process, and use carnosine as its sole source of L-histidine. Future work will involve co-culturing of *L. reuteri* with human ileal enteroids to determine the effects of *L. reuteri* and histamine metabolism on the human ileal epithelium. I will

Mucosal disease activity may predict therapeutic response to fecal microbiota transplantation in pediatric ulcerative colitis

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BACKGROUND: Fecal microbiota transplantation (FMT) is an emerging alternative treatment option for ulcerative colitis (UC). Recent trials showed variable efficacy of limited FMT series in treating UC. We explored the clinical and metagenomic responses to intense serial FMT treatments in pediatric UC patients with mild to moderate disease severity.
METHODS: Seven patients (9 treatment courses) with immunotherapy dependent pediatric UC were studied. These patients enrolled into an FMT trial with a weaning course of rectally (colonoscopy and retention enemas, Tx=37) administered FMTs during the withdrawal of their immunotherapy. Filtered, frozen and thawed donor stool specimens were utilized. Adverse events, clinical and endoscopic disease activities were monitored throughout the protocol. Fecal microbiomes were analyzed by massively parallel pyrosequencing of the V3V5 regions within the bacterial *16S rRNA* gene.
RESULTS: Patients (n=6) who had endoscopic remission or mild mucosal disease variably tolerated the treatments and the withdrawal of their immunotherapy while maintaining clinical remission. However, subjects with moderate to severe endoscopic disease (n=3) developed moderate to severe symptoms and had to be excluded from the trial within 10 days of initiation. The length of clinical remission negatively correlated (r=-0.78, p=0.013) with mucosal disease activity at the initiation of FMTs. Metagenomic stool studies revealed complex bacterial composition changes upon serial FMTs.
CONCLUSION: Our findings underscore the importance of mucosal disease staging prior to considering FMT as a primary treatment modality for UC. Patients with medically induced colonic mucosal remission or mild endoscopic disease may benefit the most from FMTs. ClincialTrials.gov number: NCT01947101 (IND#15734)

Study ID	G	R	Eth	Age (y)	Tx prior to FMT	FMT specimens	Treatment # (rescue enemas)	Mayo score before FMT	Mayo score after 12 week FMT	Days spent in remission
P001	M	W	NH	16	IFX	D001	28 (2)	0	0	261
P002*	M	W	NH	15	6-MP	D001	25	1	0	159
P003#	F	W	H	14	PRED	D001	22	0	0	105
P004*	M	W	NH	16	PRED	D001	32 (1)	1	0	204
P005	F	W	NH	18	PRED	D002	10	2		10
P006#	F	W	H	15	PRED	D001	17 (1)	1 (P)		21
P007	M	W	NH	20	PRED	D001	9	2		9
P008	M	W	NH	17	PRED	D001	2	3		2
P009	M	W	NH	13	PRED	D001	24 (5)	1 (P)		58
D001	M	A/I	NH	37						
D002	F	A/I	NH	30						

A/I: Asian/Indian; Eth: Ethnicity; F: Female; G: Gender; H: Hispanic; IFX: infliximab; M: Male; NH: non-Hispanic; PRED: prednisone; P: pseudopolyp; R: Race; W: White; 6-MP: 6-mercaptopurine. Two patients were enrolled twice a year apart (* and # indicates the same patient). We calculated pseudopolyps to add 0.5 to the endoscopic Mayo score.

Lactobacillus reuteri as a biotherapeutic system for the delivery of antimicrobial peptides.

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Nowadays, bacterial delivery systems are a popular tool to supply recombinant proteins or peptides to the gastrointestinal tract (GIT) for therapeutic purposes. Lactic acid bacteria (LAB), particularly *Lactococcus lactis* are being explored for the treatment of diseases such as inflammatory bowel disease with promising therapeutic effects. Despite these results, *L. lactis* has some features that limit its use as a therapeutic delivery system (TDS); such as being a bacterium isolated from cheese and its inability to survive in some gastrointestinal compartments. *Lactobacillus reuteri* ATCC PTA 6475 is a human derived strain with probiotic properties that has not been associated with any pathological process. These characteristics, along with the availability of genetic tools, such as recombineering and CRISPR/Cas9, that allow precise manipulation of its genome with high efficiency, make *L. reuteri* a promising candidate for the delivery of therapeutic peptides. Regenerating islet-derived protein 3 alpha (Reg3α) is an antimicrobial peptide, continuously secreted to the lumen of the human GIT, which has activity against several pathogens including Vancomycin resistant enterococci (VRE) and *Listeria monocytogenes.* The goal of our research is to construct a *L. reuteri* strain that efficiently delivers Reg3α to the GIT and can be used as a therapeutic tool for the treatment of several gastrointestinal diseases including listeriosis and VRE. For this purpose, we will engineer an *L. reuteri* strain that appropriately secretes Reg3α *in vitro* under gastrointestinal conditions, and will be used to determine the therapeutic effect of our TDS. Preliminary results show that 95% of the total produced protein is soluble, and approximately 70% is secreted to the extracellular medium. Currently we are working on achieving a suitable expression of Reg3α and demonstrating its antimicrobial activity *in vitro.* Once the expression of the protein is optimized we will test the therapeutic effect of our *TDS in vivo* by using the ligated ileal loops model of VRE .

Infants with short bowel syndrome (SBS) have decreased intestinal bacterial richness and diversity

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Background: SBS is a common cause of gastrointestinal mortality and morbidity in neonates. Though interventions such as antibiotics and probiotics are commonly used in SBS, the nature and evolution of intestinal microbiome in infants with SBS is unknown.

Objective: To describe the profile and evolution of intestinal microbiome in infants with SBS

Design/Methods: Cases included hospitalized SBS infants who underwent a surgical intervention for either a congenital or an acquired gastro-intestinal condition, and required parenteral nutrition for over 4 weeks. Controls were gestational age-matched hospitalized infants without gastrointestinal morbidities. Stool samples were collected at a weekly interval, till discharge from the hospital. DNA was extracted using the PowerMag Microbiome isolation kit (MoBio) and the bacterial V4 region of 16s rRNA gene was amplified by PCR and sequenced on the MiSeq (Illumina) platform. Sequencing reads were analyzed using the QIIME, and the SILVA database was used for operational taxonomic unit (OTU) classification.

Results: 255 stool samples were analyzed from 16 cases and 21 controls. Cases included the following infants: 4 gastroschisis, 5 small intestinal perforations, 1 necrotizing enterocolitis (NEC), 4 NEC & perforation, 1 volvulus and 1 jejunal atresia. Cases showed both decreased amount of OTUs found, and diversity of microbiome. Decreased diversity though not statistically significant, was associated with the following: birth by Cesarean section, absence of oral feeds, use of antibiotics, and use of breast milk in comparison to formula when orally fed. Within the SBS group, infants with complicated gastroschisis and NEC demonstrated the least diversity. At the level of the phylum, SBS infants showed decreased abundance of Firmictues (lachnospiraceae and lactobacillus) and greater abundance of Proteobacteria (Pseudomonas and Escherichia-Shigella).

Conclusions: We have demonstrated that infants with SBS have decreased richness, diversity and evenness of intestinal microbiome. Significantly, within the group of SBS, the diversity, evenness and abundance decreased further when associated with complications. SBS group showed greater abundance of Proteobacteria. Defining the intestinal microbiome in infants with SBS, will refine the existing intestinal microbiome-altering therapies and promote development of new strategies to improve outcomes.

The role of GABA in susceptibility to *Clostridium difficile* infection

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Clostridium difficile is a leading cause of nosocomial infection in the U.S. and represents a major health problem for hospitals and long-term care facilities. Despite an established correlation between the disruption of the gut microflora and the development of *C. difficile* infection (CDI), the mechanism of CDI susceptibility and recurrence is not well understood. Here we demonstrate that patients who develop CDI have significant antibiotic-associated shifts in stool microbiome and metabolome signatures that are functionally associated with increased L-arginine conversion to γ-aminobutyric acid (GABA). Elevated stool GABA and zolpidem use (a GABAeric drug) were predictive of disease susceptibility in patients and in experimental CDI models, and potentiated *C. difficile* toxin virulence via activation of GABA receptor A signaling. Stool GABA levels correlated with closely related GABA-producing Clostridial spp. Further investigation demonstrated that luminal GABA potentiated microbial dysbiosis during CDI and increased mucosal adherence of *C. difficile*. In summary, elevated microbial GABA production constitutes a significant new risk factor in CDI pathogenesis and represents a novel disease target for therapeutic intervention.

Epigenetic and transcriptional regulation of inflammation in IL-10-/- mouse

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Background Epigenetic modifications, exemplified by DNA methylation and histone methylation and acetylation, contribute to the regulation of gene expression. Abnormalities in these patterns of epigenetic modifications have been associated with disease processes, particularly cancer. However, the contribution of epigenetics to inflammatory bowel disease (IBD) is less known. Further, recent next-generation sequencing analyses uncovered mutations in interleukin-10 (IL-10) and the IL-10 receptor genes that associate with early onset of ulcerative colitis (UC) in children. Taken together with the IL-10 knockout (IL-10-/-) mouse model that develops chronic intestinal inflammation, it is clear that IL-10 signaling is essential to maintaining mucosal homeostasis. In concert with these observations, our previous studies identified *Rc3h1* (Roquin-1), a RING finger ubiquitin ligase with immune modulatory and RNA stability functions, as a potential player in the IL-10 regulatory pathway. Given this complex etiology of IBD, we postulated that distinct epigenetic markers may demarcate individuals that are predisposed to developing IBD. To examine the epigenetic markers associated with defective IL-10 signaling, we investigated the epigenetic profile of *Rc3h1* in mucosal biopsies from IL-10-/- mice.

Methods Using the IL-10-/- mouse model, we examined the epigenetic markers associated with intestinal inflammation. In these studies, chromatin immunoprecipitation (ChIP) followed by qPCR was used to obtain a detailed profile of methylation and acetylation at the *Rc3h1* locus in the mucosal tissue and cells of IL-10-/- mice.

Results ChIP analysis revealed that the *Rc3h1* locus had increased methylation and reduced ubiquitylation in the IL-10-/- mice. In contrast, the *Rc3h1* locus is poorly methylated and highly ubiquitylated in BALB/c mouse mucosal biopsies.

Conclusion Our data indicate that the *Rc3h1* locus is transcriptionally silenced during inflammation and suggest a fundamental role for *Rc3h1* in mucosal immune homeostasis. They further indicate that a lack of IL-10 signaling, as evidenced in the IL-10-/- mice, has significant epigenetic effects that may contribute to colitis.

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Characterization of the perinucleus in colon cancer cells

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More than fifty thousand men and women in the U.S. will prematurely succumb from colon cancer this year. It is estimated that only about 4 of 10 cases will be diagnosed at an early stage, when current treatments are most likely to be successful, and better approaches are needed. The etiology of malignant transformation is enigmatic, but appears multi-factorial and includes genetic transformations[1] many of which may be propagated by chronic inflammatory conditions,[2,3] such as perinuclear viral accumulation.[4,5] Until now, an investigation of the role of the perinucleus has been restricted by the absence of any isolation method.

We developed an isolation technique on the basis of the perinucleus' unique, compact organization, and we found that it contains approximately 17% of the total proteins of the mammalian cell, which is almost half of the proteins of nuclei.[6] Using four different normal and cancer cell lines, we demonstrated that the perinucleus' composition is highly dynamic. In this fraction we detected the oncoprotein, Ras, previously ascribed to the plasma membrane, and Src onocoprotein. Moreover, the translocation of the p53 tumor-suppressor protein to the perinucleus in immortalized MEF cells was shown to correlate with the translocation of p53-stabilizing protein, nucleophosmin (B23) to the perinucleus. We showed that the rate of fast- and slow-growing colon cancer cells depends upon the regulation of protein biosynthesis in the perinucleus, but not in the cytoplasm. Application of transmission electron microscopy and deconvolution microscopy confirmed distinct structural properties of the perinucleus.

On the basis of our results, the concept of the perinucleus is advanced as a formal, identifiable, structurally dynamic organelle. Taken together, the perinucleus maintains nuclear integrity, regulates protein biosynthesis, appears to contribute to gene expression and points to potentially new therapeutic targets. Investigation of the role of the perinucleus in malignant transformation appears highly important in the understanding of colon cancer progression. The characterization of the perinucleus, described herein, may open up broad, new research opportunities that transcend colon cancer biology, possibly including hepatocellular carcinoma, Barrett's transformation, inflammatory bowel disease and biliary atresia.

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Reuterin – Investigation of a novel signaling mechanism in gut-microbial health

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Background The most prolific cause of bacterial-induced diarrhea in the U.S. is infection by *C. difficile*. Up to one million cases are reported annually at a cost >$3.5 billion, with rates in some hospitals approaching 40%. Despite a known inverse correlation between the protective gut microbiota and the development of symptoms in infected patients, there is still a major gap in our understanding of how host bacteria protect against this pathogen. We have identified a new, potent antimicrobial defense mechanism targeting *C. difficile*, which functions as the physiological corollary of therapeutic inhibitors currently being developed to treat CDI.

Results Metagenomic analysis of stool specimens from CDI patients showed the relative abundance of *Lactobacillus* ssp. to be significantly decreased in patients who subsequently developed a recurrent episode. As a genus, *Lactobacillus* spp. are intrinsically resistant to antibiotics making this a resilient treatment option for CDI. Particularly, *L. reuteri* is resistant to vancomycin (MIC 256 μg/mL), fidaxomicin (MIC >32 μg/mL), and metronidazole (MIC >256 μg/mL), which are commonly used in CDI therapy. *C. difficile* growth *in vitro* is inhibited by *L. reuteri*, and mutant strains unable to produce the antimicrobial, reuterin, do not inhibit *C. difficile* growth. Reuterin (3-hydroxypropionaldehyde) production by *L. reuteri* requires the horizontally acquired *pdu-cbi-hem-cob* cluster and glycerol. We demonstrated that the combination of *L. reuteri* and glycerol is highly effective and selective against *C. difficile* in a human microbiota bioreactor model. Furthermore, administration of either glycerol or purified reuterin alone to bioreactors did not inhibit *C. difficile* expansion, providing strong justification for using *L. reuteri* and glycerol in animal models and patients. Using mass spectrometry, we demonstrate that reuterin forms previously unappreciated posttranslational modifications of target proteins that are vital for *C. difficile* survival, *e.g*. surface protein SlpA. Finally, we demonstrated *C. difficile* killing in the gut lumen following oral *L. reuteri* and glycerol administration, allowing development of a prototypic therapeutic concept targeting microbial infection.

Conclusions Metagenomic analysis of stool specimens from CDI patients revealed a decreased abundance of *Lactobacillus* spp. in patients with recurrent disease, which led us to screen probiotic *Lactobacillus* ssp. known to be safe in humans. We identified *L. reuteri* as an intrinsically antibiotic-resistant strain with greater cytotoxicity to *C. difficile* than vancomycin and fidaxomicin. Microbial community analysis in a bioreactor supported *L. reuteri* and glycerol as a potential therapy targeting CDI. As part of a DDC Pilot Award, we aim to characterize this previously unappreciated antimicrobial mechanism, and to exploit this novel finding to develop prototypic adjunctive treatments for CDI.

Ablation of ghrelin receptor attenuates high fructose corn syrup (HFCS)-induced adipose inflammation and insulin resistance

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Adipose inflammation and insulin resistance play causal roles in type 2 diabetes. High fructose corn syrup (HFCS) is the most-used sweetener in the United States, substantially replacing table sugar. Some studies have suggested that HFCS consumption correlates with obesity and insulin resistance, while others are in disagreement. Due to conflicting and insufficient scientific evidence, HFCS continues to be used as the primary sweetener in our food supplies. In this study, we compared the metabolic effects of mice fed regular diet, high fat diet, or regular diet supplemented with 8% HFCS in drinking water (to mimic soft drinks). As expected, high fat diet-fed mice consumed the most calories, and showed the highest weight gain and fat deposition. Surprisingly, HFCS-fed mice exhibited most severe insulin resistance, which was disproportionate to calorie intake and body fat content. Adipose tissue macrophages (ATMs) play an important role in the pathogenesis of obesity and insulin resistance. Here we show that similar to high fat diet; HFCS triggered a robust increase of both pro-inflammatory ATMs ($F4/80^+CD11c^+$) and anti-inflammatory ATMs ($F4/80^+CD11c^-$) in visceral fat. Remarkably, however, the anti-inflammatory ATMs were much less abundant in HFCS-fed mice than in high fat-fed mice. Ghrelin is an orexigenic hormone, promoting adiposity and insulin resistance. We found that deletion of ghrelin receptor growth hormone secretagogue receptor (GHS-R) ameliorates HFCS-induced adipose inflammation, insulin resistance and liver steatosis. Thus, HFCS consumption has detrimental effect on metabolism beyond the calories associated with it, and GHS-R antagonists may represent novel therapeutic option for preventing/treating inflammation and insulin resistance.

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Obeticholic acid (INT-747) confers disease protection against *Clostridium difficile* infection.

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Background: *Clostridium difficile* is the leading cause of bacterial diarrhea in the U.S. Although therapy for *C. difficile* infection (CDI) is available, conventional drugs are associated with high recurrence rates, and newer, targeted antibiotics can also result in treatment failure. Novel treatment options are therefore greatly needed for this serious disease. Farnesoid X receptor (FXR), a bile acid receptor, is known to ameliorate intestinal inflammation, and bile acids play an important role in *C. difficile* virulence. Thus, we investigated whether the synthetic bile acid analogue and potent FXR agonist obeticholic acid (INT-747) is protective against CDI.

Methods: The clinical efficacy of daily INT-747 (10-50 mg kg^{-1} day^{-1}) was tested in an established mouse CDI disease model using wild type C57bl and FXR (-/-) genetically deficient strains. *In vitro,* we assessed the INT-747 minimum inhibitory concentration as well as the effect of INT-747 on spore germination of *C. difficile* VPI1640, 630 and clinical BI/027/NAP1 strains.

Results: Oral INT-747 demonstrated good clinical efficacy in C57bl mice infected with 10^3 VPI1640 or NAP1 *C. difficile* spores. Whereas vehicle treated animals lost > 10% body weight after 48 hr, INT-747 treated animals maintained a baseline weight that was comparable to uninfected mice. Surprisingly, INT-747 exerted a similar protective effect in FXR (-/-) deficient mice, suggesting that it may directly target *C. difficile*. This was confirmed by *in vitro* assays where INT-747 inhibited spore germination with similar potency to chenodeoxycholic acid (CDCA) [K_i for CDCA 131 $\pm$ 10 µM *vs* 215 $\pm$ 46 µM for INT-747.] Furthermore, INT-747 inhibited *C. difficile* growth in the low µM range.

Conclusion: INT-747 is currently receiving much attention as a treatment for diverse liver diseases as well as insulin resistance. Here we demonstrate a novel off-target application for INT-747 as preventative therapy for CDI, via direct inhibitory action on *C. difficile* spore germination and growth. With current FDA fast-track designation, INT-747 may represent an attractive new therapeutic tool in the fight against *C. difficile*.

Induction of glucagon-like peptide-1 production from L cells by human microbial strains

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Glucagon-like peptide-1 (GLP-1) is an incretin secreted by enteroendocrine L cells which line the gastrointestinal epithelium. GLP-1 has numerous functions, including promoting insulin secretion, insulin sensitivity and β-cell mass, while inhibiting glucagon secretion, β-cell apoptosis, gastric emptying and appetite. As such, GLP-1 analogs are currently being used as therapeutics for Type II Diabetes Mellitus (T2DM); unfortunately, the current formulations have important limitations. Recent work has demonstrated that the gut microbiota plays important roles in T2DM, as well as cardiovascular disease, obesity, hypercholesterolemia and non-alcoholic fatty liver disease. Indeed, the human gut microbiota has been shown to alter hormone secretion, including GLP-1. Microorganisms that are capable of promoting GLP-1 production may provide a successful therapeutic approach to combat T2DM and obesity. The research objective is to identify human associated bacteria that have the ability to stimulate GLP-1 production by gastrointestinal L cells. Over 1000 microbial strains were isolated from human fecal samples, breast milk, and healthy human colon and ileum biopsies. *In vitro* screening investigations were performed using NCI H716 human intestinal L cells. Bacterial supernatants were incubated with NCI H716 cells for 2 h followed by quantification of GLP-1 levels by ELISA. From the screening of 650 human microbial isolates, 45 strains capable of increasing GLP-1 levels have been identified and require further investigations. Additional experiments are needed to elucidate the mechanism(s) of action behind the observed increases in GLP-1 levels. Ultimately, a safe and efficient microbial therapeutic formulation for combatting obesity and T2DM is desired.

HGF and Met support mouse enteric nervous system development, the peristaltic response, and intestinal epithelial proliferation in response to injury.

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Although many enteric neuron subtypes are known, factors providing trophic support to these neurons remain poorly understood. We tested the hypothesis that hepatocyte growth factor (HGF) and the HGF receptor MET might support some types of enteric neurons and found that HGF and MET are expressed in the fetal and adult enteric nervous system (ENS). *In vitro*, HGF increased enteric neuron differentiation and neurite length when added to cultured ENS precursors, but only if vanishingly small amounts (1 pg/mL) of glial cell line-derived neurotrophic factor (GDNF) were included in the media. HGF effects on neuron number were blocked by phosphatidylinositol-3 kinase (PI3K) inhibitors and by MET blocking antibody. These inhibitors also reduced neurite length as did MEK inhibitor. In adult mice, MET was restricted to a subset of calcitonin gene related peptide (CGRP) immunoreactive (IR) neurons within the myenteric plexus that are thought to be intrinsic primary afferent neurons (IPANs). A conditional inactivating mutation in the MET kinase domain (*Met$^{fl/fl}$; Wnt1Cre+* mice) caused markedly reduced peristalsis in response to mucosal deformation, but not in response to radial muscle stretching. This mutation also caused a dramatic loss of MET-IR neurites in the myenteric plexus detected with an antibody to the expressed MET extracellular domain. Finally, *Met$^{fl/fl}$; Wnt1Cre+* mice had more bowel injury and reduced epithelial cell proliferation compared to WT animals after dextran sodium sulfate (DSS) treatment. These results suggest that HGF/MET signaling is important for development and function of a subset IPANs and that these cells critically regulate intestinal motility and epithelial cell proliferation in response to bowel injury.

Perfusion with celecoxib decelerates the rates of PGE_2 production in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-treated rat colon

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Purpose: The cyclooxygenase-2 (COX-2) protein is often expressed in inflamed tissues and several malignancies including colorectal cancer (CRC). 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis in rat colon is frequently used as a model for inflammatory bowel disease (IBD), in which COX-2 is substantially induced. In this study, celecoxib, a selective and potent inhibitor of COX-2, was assessed for its effect on COX-2 activity in rat colon with inflammation induced by TNBS.

Method: 30mg TNBS in 200μL 50% ethanol was administrated to the colon of Sprague Dawley (SD) rats. 12 hours later, the rats were anesthetized and the segments of proximal colon were cannulated and perfused with 0.5mL/min hank's balanced salt solution (HBSS) containing vector (0.1% DMSO) or 0.1, 1 and 10μM celecoxib (n=4~6 for each group). The perfusate was collected from the anus per 20 minutes for 3 hours and then concentrated by solid-phase extraction procedures. The contents of prostaglandin E_2 (PGE_2) in the concentrate were determined by liquid chromatography-tandem mass spectrometer (LC-MS/MS) as the indicator of COX-2 activity in inflamed colon tissue.

Result: The concentrations of PGE_2 in the perfusate decreased during the perfusion period and its attenuation rate could be appropriately described by a first-order equation. In the control group perfused with blank HBSS, the attenuation rate constant K was determined to be $0.005\pm0.001 min^{-1}$. In the groups perfused with 0.1, 1 and 10μM celecoxib, K was determined to be 0.006±0.002, 0.011 ± 0.003 and $0.020\pm0.002 min^{-1}$, respectively. In the control group the half-life of PGE_2 attenuation was 156±49min, while in the groups perfused with 0.1, 1 and 10μM celecoxib the half-life was 127±36, 70±19, and 37±4min, respectively. Compared with the control group, perfusion with 1 and 10μM celecoxib significantly decreased the half-life of PGE_2 attenuation in the perfusate ($P<0.001$ and $P<0.00002$, respectively).

Conclusion: Perfusion with celecoxib decelerated the PGE_2 production in TNBS-induced colitis by inhibiting the activity of COX-2 in inflamed rat colon, and the effect is concentration-dependent. This suggests that this colon perfusion model may be used an in vivo screening model of COX-2 inhibitors.

Feeding *Lactobacillus reuteri* DSM 17938 produces local and systemic anti-inflammatory effects in Treg-deficient Scurfy mice

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Background: *Lactobacillus reuteri* DSM 17938 (LR17938) has been shown to have anti-inflammatory activities in animal models of necrotizing enterocolitis (NEC), associated with reduced levels of ileal cytokines via a TLR-4-dependent pathway, while differentially modulating effector/memory T (Tem) and regulatory T (Treg) cell balance in the intestinal mucosa. Foxp3$^+$Treg cells modulate the function of multiple immune cell types. Loss of Tregs causes lethal, CD4$^+$T-driven autoimmune disease in the Scurfy, sf mouse and in humans with IPEX syndrome (Immune dysregulation, polyendocrinopathy, enteropathy, X-lined). Sf mice generally die of a lymphoproliferative syndrome and severe multi-organ inflammation on day of life (DOL) 21-28. When we fed LR17938 to sf mice by gavage, their survival was significantly increased. Mechanisms by which LR17938 contributes to prolong survival have not been investigated.
Aims: To determine if oral feeding of LR17938 to sf mice changes activated CD4$^+$T cell in intestinal mucosa, spleen and peripheral blood, and if LR17938 inhibits Th1/Th17 differentiation.
Methods: Scurfy mice were obtained by breeding pairs of Foxp3sf heterozygous female and C57BL/6J normal male (Jackson Labs). Because the Foxp3 gene is on the X chromosome, 25% of the offspring will be hemizygous males with the mutation (sf). Male sf mice generally develop the scurfy phenotype (scaly, crusted skin on the tail, poor growth) on DOL 13-15. Once sf phenotype was recognized, mice were fed LR17938 (10^7 CFU/day) by gavage, daily for 7 days. Immune cells isolated from ileum, mesenteric lymph node (MLN), spleens and peripheral blood were labeled with anti-CD4/CD44 to look for cell activation and with anti-CD45Rb to quantify inflammatory Tem cells by flow cytometry. Naïve CD4$^+$T cells isolated from spleen of wild-type (wt) mice were *in vitro* cultured and induced to differentiate to Th1 and Th17 by adding specific cytokines/antibodies in the presence or absence of LR17938 (ratio of 1:1 (LR : cell)). After 5 days, cells were re-stimulated by PMA/Inomycin with brefeldin for 5h, then stained for CD4, intracellular IFNγ (Th1), and IL17A (Th17) and analyzed by flow cytometry.
Results: Sf mice showed significantly increased activated CD4$^+$T cells and inflammatory Tem cells in intestinal mucosa compared to wt mice. Oral feeding LR17938 to sf mice significantly reduced the expansion and activation of CD4$^+$T cells and inflammatory Tem cells in the intestine and MLNs. Systemic anti-inflammatory effects of LR17938 were also observed in sf mice. Sf splenocytes contain a highly expanded population of activated CD4$^+$T and Tem cells. Oral feeding of LR17938 reduced the number of CD4$^+$T cells. Importantly, in cultured splenocytes, the percentages of proinflammatory IL17A$^+$CD4$^+$T cells were decreased in LR17938-fed sf mice. In addition, feeding LR17938 to sf mice decreased the activation of CD4$^+$T and Tem cells in peripheral blood. LR17938 directly inhibited the differentiation of both Th1 and Th17 cells from naïve CD4$^+$T cells *in vitro*.
Conclusions: The local and systemic anti-inflammatory effects of probiotic LR17938 were shown, demonstrating that LR17938 inhibited the activation of CD4$^+$T cells and their differentiation to Th1/Th17 cells, despite the complete absence of Foxp3$^+$Treg cells. LR17938 may be beneficial for human autoimmune and inflammatory diseases.

Texas Medical Center Digestive Diseases Center
6th Annual Frontiers in Digestive Diseases Symposium:
Frontiers of Inflammatory Bowel Diseases in 2015

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