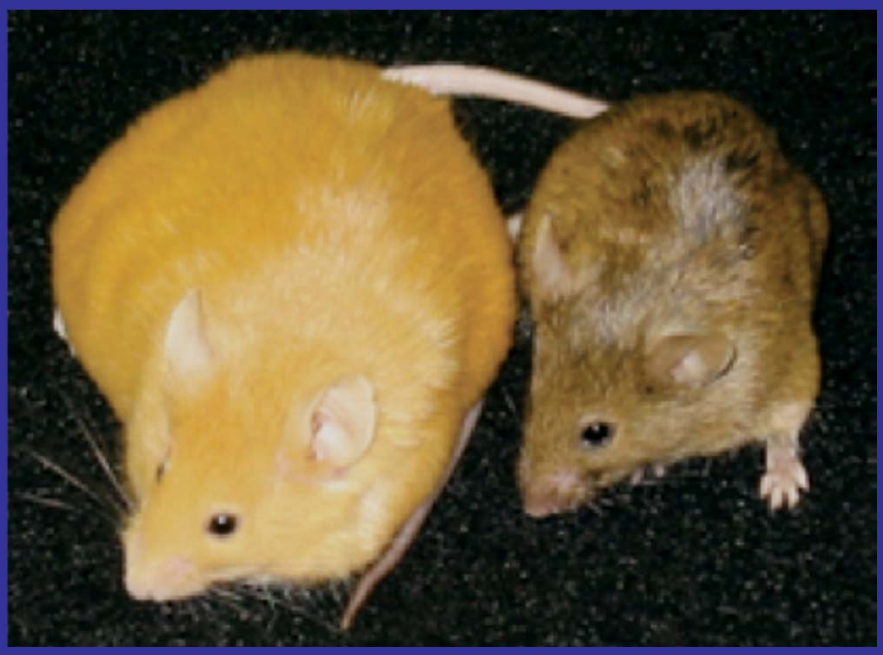
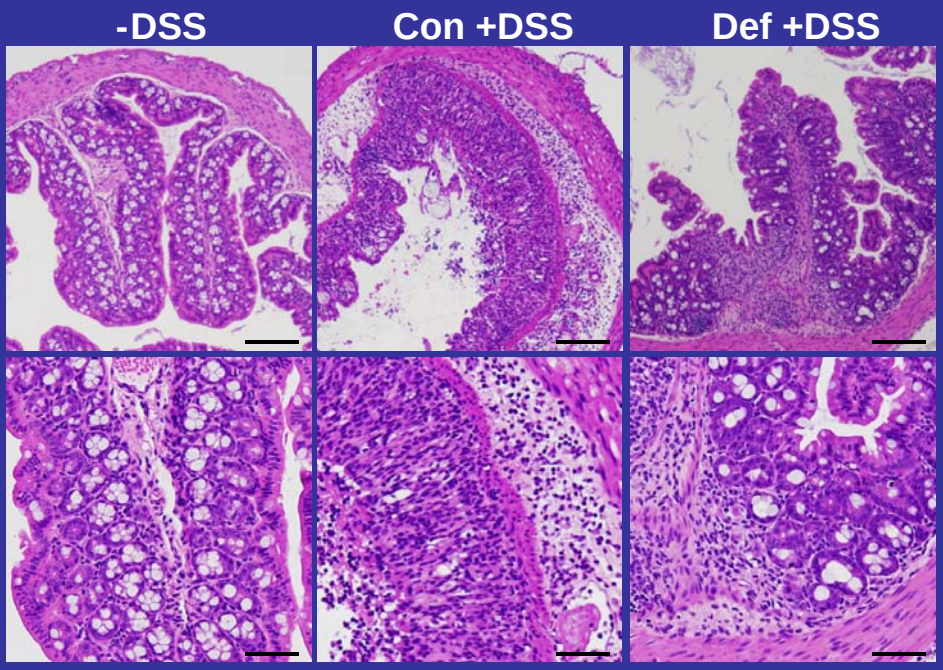
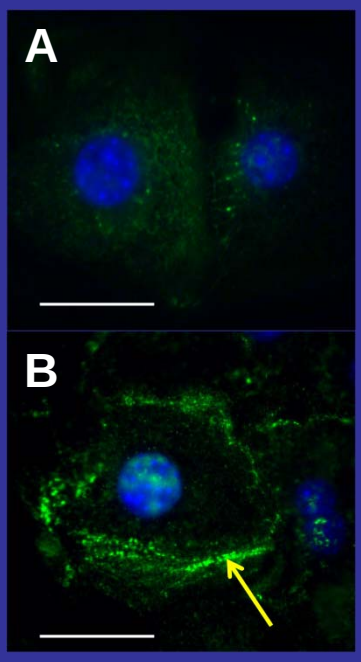
Texas Medical Center Digestive Diseases Center
3rd Annual *"Frontiers in Digestive Diseases"* Symposium:
Epigenetics in GI Health and Disease







Monday, March 7, 2011
Hickey Auditorium, UT MD Anderson Cancer Center
Houston, Texas

Texas Medical Center Digestive Diseases Center
2nd Annual *"Frontiers in Digestive Diseases"* Symposium: Infection in GI Health and Disease

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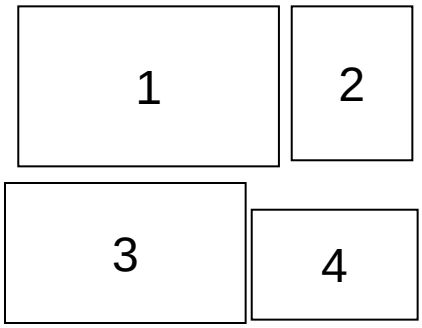

1. P-47. Wang W. *In vivo* imaging of a liver cancer xenograft with 18F-FDG, RGD and MMP imaging agents. (A) The mouse color image; (B) 18F-FDG PET image; (C) RGD optical image; (D) MMP optical image; (E) Merged18F-FDG PET/RGD optical image; (F) Merged 18F-FDG PET/MMP optical image; (G) Merged MMP/RGD optical image; (H) Merged 18F-FDG /MMP/RGD image; (I) Merged 18F-FDG PET/CT (skeleton) image; (J) Merged RGD/CT (skeleton) image; (K) Merged MMP/CT (skeleton) image; and (L) Merged 18F-FDG/RGD/MMP/CT (skeleton) image. The color image shows the tumor location.18F-FDG PET imaging demonstrates high glucose uptake in the tumor. RGD optical imaging indicates blood vessel formation in the tumor region. MMP optical imaging further exhibits MMP imaging agent bound to the tumor.

2. P-18. Hertel P. Consecutive sections of BA mouse liver 4 days post-inoculation with saline (left panels) or 10^7 pfu live rotavirus (right panels). Tissue was stained with antibody against rotavirus (A, B), CK-19 (C, D, a marker of bile duct epithelium), or osteopontin (E, F). Uninfected, non-inflamed bile ducts in saline-treated pups stain for osteopontin. However, areas of rotavirus and CK-19 staining are surrounded by dense inflammation and show increased OPN-stained cells.

3. P-51. Youmans B. Heatmap showing the abundance of bacteria at the Family level in Healthy, ETEC-associated Travelers' Diarrhea (ETEC-TD), and Non-ETEC-associated Travelers' Diarrhea samples (Non-ETEC-TD). ETEC-associated Travelers' Diarrhea samples are further divided into Labile toxin positive (LT+), Stable toxin positive (ST+), and Labile and Stable toxin positive (LT+/ST+). Fewer percentage of reads is represented by light blue and higher percentage of reads is represented by orange.

4. Conner ME. Rotavirus antigens are associated with follicle-associated epithelium and subepithelial dendritic cells early after viral inoculation. Mice were orally inoculated with 10^5 ID50 of rotavirus ECwt and then Peyer's Patch sections examined by immunofluorescence. Rotavirus is shown in green (A-C), CD11c (dendritic cell marker) is shown in red (A-D), and cell nuclei in blue (B). Overlap of Rotavirus and CD11c is shown in yellow in the merged images (arrows). Section stained with anti-CD11c and control polyclonal antibody is shown in (D). A, C and D 200X magnification. B 400X magnification.

Texas Medical Center Digestive Diseases Center
2nd Annual *"Frontiers in Digestive Diseases"* Symposium:
Infection in GI Health and Disease

A G E N D A
Monday, April 12, 2010
Marriott Medical Center Hotel, Grand Ballroom
6580 Fannin, 713-796-0080
Houston, Texas

Registration	
8:00am	***Coffee and Continental Breakfast***
8:30am	***Welcome:*** Mary K. Estes, Director, TMC Digestive Diseases Center
1st Session	***Theme:*** **Mechanisms of Infection and Injury in the Liver and Pancreas** ***Moderator:*** **Sundararajah Thevananther, BCM**
8:45 – 9:15am	Hashem El-Serag, BCM *Epidemiology and Burden of Hepatitis C in the United States*
9:15 – 9:30am	Paula Hertel, BCM *Inflammation and Fibrosis in Biliary Atresia: Dual Roles for Osteopontin?*
9:30 – 10:15am	**Guest Speaker: Stanley Lemon**, University of Texas Medical Branch/Galveston *miR-122 in Hepatitis C Virus Infection*
10:15 – 10:30am	***Morning Coffee Break***
10:30 – 10:45am	Shumei Song, MDACC *The Role of Galectin-3 in the Pathogenesis of Pancreatic Cancer*
10:45 – 11:00am	Rhomi Ghose, UH *Regulation of Hepatic Drug Metabolizing Enzymes in Inflammation*
11:00 – 11:30am	Betty Slagle, BCM *Molecular Mechanisms of Hepatitis B Virus Replication*
11:30 – 1:30pm	***Lunch & Poster Viewing*** *Poster Judging: Robert Bresalier, Milton Finegold, and Claudia Kozinetz*
2nd Session	***Theme:*** **Mechanisms of GI Infection and Injury** ***Moderator*****: Marc Rhoads, UTHSC**
1:30 – 2:15pm	**Guest Speaker: Phillip Smith**, University of Alabama at Birmingham *Mucosal Responses to HIV-1 and CMV in the Human Small Intestine*
2:15 – 2:30pm	Joe Hyser, BCM *Disruption of Intracellular Calcium Homeostasis by a Viroporin Domain in the Rotavirus Enterotoxin NSP4*
2:30 – 2:45pm	Yuying Liu, UTHSC *Lactobacillus Reuteri Strains Reduce Inflammation in Experimental Necrotizing Enterocolitis*
2:45 – 3:15pm	Margaret Connor, BCM *Role of Gut Associated Lymphoid Tissue (GALT) in Rotavirus Infection and Immunity*
3:15 – 3:30pm	David Graham, BCM *New Approaches to Treatment of H. Pylori Infection*
3:30 – 4:00pm	Herbert DuPont, BCM and UTHSC *Diarrhea Caused by Enterotoxigenic Eschichia coli - From Travelers to Food-borne Disease in the U.S.*
4:00pm	**Closing Remarks**: Mark Gilger, BCM *(Announcement of poster award winners)*
4:00 – 4:45pm	**Social Mixer** – Please join us and continue discussion with colleagues

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P-10.	David Graham	Treatment success with dual proton pump inhibitor plus amoxicillin *H. pylori* therapy or PPI, amoxicillin, clarithromycin triple therapy
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P-12.		2. Can Lipocalin 2/NGAL in exocrine pancreatic secretions distinguish chronic pancreatitis from pancreatic cancer?
P-13.		3. Neutrophil gelatinase-associated lipocalin inhibits proliferation of human esophageal adenocarcinoma cells both *in vitro* and *in vivo*

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☆ Denotes past Pilot/Feasibility awardee

☆☆ Denotes 2010 Pilot/Feasibility awardee

Texas Medical Center Digestive Diseases Center
2nd Annual *"Frontiers in Digestive Diseases"* Symposium:
Infection in GI Health and Disease

Anti-inflammatory properties of methylthioadenosine in experimental colitis

Nancy Benight[1-2], Barbara Stoll[2], Patrycja Puiman[3], Caroline Bauchart-Thevret[2], Douglas Burrin[2]
[1]Translational Biology Molecular Medicine Program, Baylor College of Medicine [2]Pediatrics, Baylor College of Medicine/Children's Nutrition Research Center [3]Pediatrics, Erasmus MC - Sophia Children's Hospital, Rotterdam, Netherlands.

Background: The methionine (Met) metabolic cycle is critical for normal cell functions. Met cycle disruption has been implicated in disease such as alcoholic liver disease (ALD) and multiple sclerosis (MS). Studies in animal models of ALD and MS have shown that the Met metabolite methylthioadenosine (MTA) has anti-inflammatory actions. Recent studies have shown that MTA's action is mediated by changes in methylation of key pro-inflammatory genes. Met metabolism may also be disrupted in inflammatory bowel disease (IBD), as indicated by elevated levels of the Met metabolite homocysteine in IBD patients. However, the role of Met metabolism and its metabolites in IBD is poorly understood. We hypothesized that administration of MTA would reduce inflammation during experimental colitis**.**

Methods: We designed a study to test the role of MTA supplementation in experimental colitis using mice treated with dextran sulfate sodium (DSS). Male C57Bl/6J mice divided into 3 groups; control (Con), DSS, and DSS+MTA. Con were healthy untreated mice. DSS and DSS+MTA received 3% DSS via the drinking water to induce colitis for 5 days. DSS+MTA also received MTA supplemented in the drinking water (150-mg/kg BW) for 2 day prior to and during all DSS days (7 days total). We measured daily weight change and scored a clinical disease activity index (cDAI). Plasma, colon, and liver collected at the end of the study. Tissues and plasma were analyzed for Met metabolite levels, myeloperoxidase (MPO) activity, gene expression changes using qPCR, and histopathology.

Results: MTA supplementation prevented weight loss and reduced the cDAI in the DSS+MTA group compared to DSS. Supplementation reduced MPO activity compared to DSS. Histological damage was reduced with supplementation. Plasma, colon, and liver MTA concentrations did not differ between the groups. Colonic S-adenosylmethionine (SAM) was reduced in both DSS and DSS+MTA compared to control, but were not different from each other. Liver S-adenosylhomocysteine (SAH) followed the same pattern, while liver SAM was increased in both groups compared to control. Plasma Met levels were reduced with DSS and restored to control levels with MTA. In the colon Met was significantly higher in the DSS+MTA mice compared to both Con and DSS. Expression levels of TNFα and iNOS were reduced in DSS+MTA when compared to DSS.

Conclusions: MTA supplementation was protective during colitis. We postulate that MTA is modulating colonic inflammation via changes in methylation status of key inflammatory genes.

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MyD88 is required for viral-induced B cell activation and intestinal IgA production.

Sarah E. Blutt[1,2], Shizuo Akira[3], Lynn Dustin[4], Margaret E. Conner[1,2]

[1] Department of Molecular Virology and Microbiology, Baylor College of Medicine
[2] Michael E. Debakey Veterans Affairs Medical Center
[3] Osaka University, Japan
[4] The Rockefeller University, New York

Background:
The role of TLR signaling in the induction of specific antibody responses is controversial and defined primarily using model antigens. Our study objective was to determine whether TLR signaling is required to generate pathogen-specific antibody responses.

Aims and Methods:
To assess whether TLR signaling is required for a viral-specific intestinal B cell responses, we examined whether B cell responses were aberrant after rotavirus infection of mice lacking expression of specific adapter proteins used in TLR signaling (MyD88, TIRAP, TRIF).

Results:
Rotavirus infection of MyD88$^{-/-}$ mice did not result in a significant induction of either B cell activation or intestinal rotavirus-specific IgA. In contrast, mice lacking TIRAP and TRIF expression had wild type levels of both B cell activation and intestinal IgA production. B cell activation was normal in mice with a defect in BCR signaling (xid) or mice expressing an OVA-specific BCR, indicating that rotavirus-induced B cell activation does not require a rotavirus-specific BCR.

Conclusions:
These findings indicate that BCR-independent signaling through MyD88 plays a critical role in pathogen induced B cell activation and the generation of pathogen-specific intestinal IgA.

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A preliminary comparison of the distal gut microbiome from HIV infected and healthy subjects

Lara M. Bull-Otterson PhD MPH[1], Bonnie Youmans[2], Yue Shang MS[2], Sarah K. Highlander PhD[1,2], Joseph F. Petrosino PhD[1,2], Kim Worley PhD[1], Lisa Armitige MD[3], Richard A. Gibbs PhD[1]
1 Baylor College of Medicine, Human Genome Sequencing Center, 2 Baylor College of Medicine, Department of Molecular Virology and Microbiology, 3 University of Texas-Health Science Center-Houston, Department of Infectious Disease

Background: There is increasing evidence that the GI microbiome plays a role in immune health and by using metagenomics, we have started to reconceive the interaction between bacteria and host. Within the last decade the requisite role of the GI tract in HIV infection and pathogenesis has become increasingly evident. HIV infects CD4+ cells preferentially from the GI tract, and CD4+ memory cells are more frequently infected within the GI tract than are peripheral blood memory cells. Furthermore, HIV viral replication is believed to play a fundamental role in perpetuating microbial translocation into the systemic circulation, suggesting that the microbiome may have significant impact on disease progression and long-term outcomes in HIV infected persons.

Aims: To examine the relationship between HIV infection and the distal colon's microbiome, we evaluated the difference in microbial community structure and diversity in HAART-naïve HIV infected patients (CD4 cell count <300 cells/ml) and compared their microbiome to HIV-negative healthy controls. Our hypothesis was that the abundance of key bacterial memberships of the fecal microbiota would be different between AIDS defined HIV positive cases and healthy HIV negative matched controls. We also believed that the abundance of the key membership bacteria would change between enrollment and 6 month on HAART in the cases.

Methods: All subjects were matched by sex, race and age. 16S rDNA from fecal samples was amplified and sequenced using standard Sanger methods (1536 clones, 3072 reads per subject). Reads were compared to RDB database to identify the organisms present, and the taxonomic groups were compared between cases and controls.

Results: We found that in all samples, the predominant bacterial classes were the Clostridia (Phylum Firmicutes) and Bacteroidetes (Phylum Bacteroidetes), and to a lesser extent Actinobacteria (Phylum Actinobacteria). There was greater variation in the number of minor groups found in the control, including representatives from Betaproteobacteria and Erysipelotrichidae, which were not identified in the HIV-infected cases. We also found that in both case groups there was an average reduction of the Bacteroidetes to half of the community membership representation that was observed in the controls and a substantial increase in Clostridia and Bacilli when compared to the controls. Most significantly, there was a clear trend in the differences between the subjects. Group A had a slightly more advanced disease and almost a log greater HIV viral load (average 5.26 log10 copies/ml) than Group B (average 4.32 log10 copies/ml). While both case groups showed drastic changes at the phylum level compared to the controls, Group A also showed both a greater reduction in Bacteroidetes and a greater increase in Firmicutes than Group B, doubling the membership of Bacilli in comparison to Group B. Similarly there was greater variation in the number of minor class groups found in Group B compared to Group A, although there was less variation than found in the controls.

Conclusions: This suggests that the shift in the GI microbial structure maybe correlated with HIV viral load in a dose-dependent manner. This is the first study to examine the relationship between HIV infection and the human distal colon's microbiome, and the first to report a possible association between HIV viral load and key microbial community membership.

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P2Y2 purinergic receptor knockout mice exhibit increased susceptibility to liver injury in a mouse model of biliary fibrosis.

Samson Cantu[1], Arunmani Mani[1], Bryan Tackett[1], Moreshwar Desai[1], Sundararajah Thevananther[1].

[1]Department of Pediatrics/Gastroenterology, Hepatology & Nutrition and Molecular and Cellular Biology, Baylor College of Medicine and Texas Children's Liver Center, Houston, TX.

Background: Extracellular ATP via the activation of P2 purinergic receptors influence multiple hepatic functions. Cellular stress and injury can induce ATP release and contribute to elevated extracellular ATP levels at the sites of injury *in vivo*. However, the functional significance of P2Y2 purinergic receptor activation during chronic liver injury is currently unexplored. Most notably, biliary fibrosis is among the well-documented causes of end-stage liver disease, which lacks effective therapies preventing disease progression.

Aims: The purpose of this study was to test the **hypothesis** that extracellular ATP and P2Y2 purinergic receptor-mediated signaling protects against hepatobiliary injury and biliary fibrosis.

Methods: Adult (8-10 week old) male wild type (C57BL6/J; WT) and P2Y2 -/- (KO) mice were fed chow or 3, 5-diethoxycarbonyl-1, 4-dihydrocollidine (DDC), for 1-3 weeks. Serum (ALT, AST, ALP, total and direct bilirubin), liver sections (H&E and Mason Trichrome), and protein extracts (Western blotting) were analyzed in order to assess the extent of cholestasis, biliary fibrosis and liver injury. Statistical analysis was performed using Student's t-test and a *P* value of <0.05 was considered significant.

Results: Serum parameters of liver injury were elevated after DDC feeding in the WT mice. Interestingly, KO mice sustained exaggerated liver injury in response to DDC feeding (1 week), as reflected by the several fold increase in serum ALT (7.7), AST (6.2), ALP (1.8), total bilirubin (3.0), and direct bilirubin (13.5) as compared to WT. Correspondingly, H&E and Mason Trichrome analysis of liver sections of DDC-fed mice (3 weeks) confirmed higher neutrophil infiltration and exaggerated ductular reaction and periductal fibrosis—characteristic features of biliary fibrosis in the KO, as compared to WT. Analysis of total liver homogenates by Western blotting revealed that the KO livers had increased activation of pro-apoptotic caspase-12 (1.8-fold) and caspase-3 (2.1-fold) as well as attenuated induction of anti-apoptotic bcl-2 (0.5-fold) in response to DDC-feeding (1 week), as compared to WT (1.0).

Conclusions: Our findings suggest that P2Y2 purinergic receptor KO livers have higher susceptibility to hepatobiliary injury in a well-established xenobiotic-induced mouse model of biliary fibrosis. Elevated serum and tissue markers of cholestatic liver injury and biliary fibrosis in the KO mice is highly suggestive of hepatoprotective roles of extracellular ATP and P2Y2 purinergic receptors during chronic liver injury, with implications for the development of targeted therapies for the management of hepatobiliary disorders.

CEA interacts with TGF-β receptor and inhibits TGF-β signaling in colorectal cancers

Hong Cao[1], Ying Li[1], Wenguo Yao[1], Wilma Jogunoori[1], Arun Thenappan[1], Lopa Mishra[2]

[1]Georgetown Univ., Washington, DC; [2]University of Texas MD Anderson Cancer Center, Houston, TX

Background: Carcinoembryonic antigen (CEA) is a tumor marker for the clinical management of colorectal cancer (CRC). The elevated blood levels of CEA are associated with metastasis and poor prognosis in CRC. There is mounting evidence that CEA enhances the metastatic potential of cancer cells. CEA increases the ability of weakly metastatic CRC to colonize the liver and to develop spontaneous hematogeneous liver and lung metastases. CEA expression has also been related with resistance to cytotoxic chemotherapy and to anoikis, a form of apoptosis caused by detachment from cell matrix. Yet the mechanism of CEA mediated metastasis is only partially understood. The TGF-β (transforming growth factor beta) signaling pathway contributes to tumorigenesis by controlling several biological processes, including cell proliferation, differentiation, migration and apoptosis. It has been reported that TGF-β regulates CEA transcription and secretion, however, little is known about the effects of CEA on TGF-β signaling.

Aims: Based on the above facts, we focused on the influence of CEA on the TGF-β signaling in both normal cells and colorectal cancer cells.

Results: Our preliminary data showed that CEA directly interacted with TGF-β receptors. Overexpression of CEA blocked TGF-β induced SMAD3 phosphorylation, SMAD3 translocation to nuclear and the downregulation of c-myc transcription. Targeting CEA with anti-CEA antibody rescued TGF-β response in CRC cell lines with elevated CEA expression, thereby restoring the inhibitory effects of TGF-β on the proliferation of these cancer cells. Finally, in animal experiment, we found that CEA enhanced survival of colorectal cancer cell in both local colonization and liver metastasis.

Conclusion: Since CEA is a well-characterized tumor-associated antigen that is frequently overexpressed in tumors, specific antibodies targeting CEA have been developed as a novel therapeutic approach for treatment of tumors expressing CEA on their surface. Based on our study, it may be helpful to combine CEA antibody and TGF-β to inhibit cancer cell proliferation and metastasis in some cases.

The role of autophagic membranes in rotavirus morphognesis

Sue E. Crawford and Mary K. Estes
Department of Molecular Virology and Microbiology, Baylor College of Medicine

Background: Rotavirus (RV) is the leading cause of severe diarrhea among infants and young children. A unique feature of RV replication involves the RV nonstructural protein 4 (NSP4). NSP4 is synthesized as an endoplasmic reticulum (ER)-specific transmembrane glycoprotein. The C-terminus of NSP4 extends into the cytoplasm and serves as an intracellular receptor for double-layered particles (DLPs) that are assembled in viroplasms, sites of virus replication. This interaction triggers the budding of DLPs into the lumen of the ER where they are transiently enveloped. The envelop is lost and the outer capsid proteins are assembled onto the particle resulting in infectious particles. Currently, the membranes used for DLP envelopment are thought to be ER membranes. We have recently shown NSP4 colocalizes with the autophagy marker LC3 in membranes, forming puncta that merge and cap viroplasms.

Autophagy is a cellular response that functions to dispose of excess or defective proteins and organelles by the formation of double-membrane vesicles, which fuse with lysosomes for degradation. Other RNA viruses such as poliovirus and rhinovirus subvert autophagy membranes for viral replication.

Aim: I hypothesize that NSP4 induces autophagic membrane formation and localizes in these membranes to function as an intracellular receptor for the assembly of infectious RV.

Results: I found that RV infection leads to LC3-I processing into the lipid-conjugated membrane-bound form of LC3-II, an early step in the formation of autophagy membranes. The yield of RV in the presence of the autophagy inhibitor, 3-methyladenine, is reduced by approximately two logs. Additionally, the yield of viral progeny in RV-infected, LC3 siRNA-transfected cells, is 41% and 36% of the yield obtained in RV-infected, control, nontargeting siRNA-transfected cells at 8 and 12 hours post infection, respectively.

Conclusions: These data suggest the induction of autophagic membranes is important for RV replication. Ongoing studies seek to elucidate the mechanism by which RV induces the formation of autophagic membranes.

Texas Medical Center Digestive Diseases Center
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Infection in GI Health and Disease

Bile acids improve the bioavailbility and bacteriostatic effect of rifaximin

Charles Darkoh[1,5], Lenard M. Lichtenberger[1,2], Nadim Ajami[3], Elizabeth J. Dial[1,2], Zhi-Dong Jiang[4,5], Herbert L. DuPont[1-6]

[1]The University of Texas Graduate School of Biomedical Sciences, [2]The University of Texas Medical School, [3]Baylor College of Medicine, [4]The University of Texas School of Public Health, Center For [5]Infectious Diseases, [6]St. Luke's Episcopal Hospital

Introduction: Diarrhea is one of the most common infirmities affecting international travelers, occurring in 20-50% of persons visiting developing regions from industrialized countries. Enterotoxigenic *Escherichia coli* (ETEC) is the most common causative agent isolated in approximately half of the cases of travelers' diarrhea. Rifaximin, a largely water-insoluble and non-absorbable (<0.4%) antibiotic that inhibits bacterial RNA synthesis, is approved for the treatment of travelers' diarrhea caused by diarrheagenic *E. coli*. However, the drug has minimal effect on bacterial flora or the infecting *E. coli* in the aqueous environment of the colon. The purpose of this study was to evaluate the bacteriostatic effect and bioavailability of rifaximin in aqueous solution in the presence and absence of physiologic concentrations of bile acids.

Methods: Methods used included growth measurement of ETEC (strain H10407), rifaximin solubility measurements, total bacterial protein determination, and assessment of functional activity of rifaximin by monitoring inhibition of bacterial β-galactosidase expression.

Results: Solubility studies showed rifaximin to be 70-120 fold more soluble in bile acids than in aqueous solution. Addition of both purified bile acids and human bile to rifaximin at sub-inhibitory and inhibitory concentrations significantly improved the drug's anti-ETEC bacteriostatic effect by 88% after 4 hours. This observation was confirmed by showing a decrease (43%) in overall amount of total bacterial proteins expressed during incubation of rifaximin plus bile acids. Rifaximin-containing bile acids inhibited (35%) the expression of ETEC β-galactosidase at a higher magnitude compared to samples that did not contain bile acids. The study provides data showing that bile acids solubilize rifaximin on a dose-response basis thereby, increasing the antibiotic's anti-ETEC bacteriostatic effect.

Conclusions: These observations suggest that rifaximin may be more effective in the treatment of infections in the small intestine due to higher concentration of bile in this region of the gastrointestinal tract than in the aqueous colon. Water insolubility of rifaximin is likely to explain the drug's minimal effects on colonic flora and fecal pathogens despite in vitro susceptibility. Our findings have important implications as a potential for the use of hydrophobic detergents and/or biocompatible compounds to improve the efficacy and antimicrobial effect of hydrophobic antibiotics

Chronic hepatitis C virus induced disruption in glucose homeostasis and insulin signaling are associated with an autophagic responsein a liver cell model

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Background: Incidence of diabetes is about three times more in patients with chronic hepatitis C virus (HCV) infection. HCV also induces insulin resistance (IR) independent of other liver diseases. Response to interferon (IFN) based therapy is reduced in the presence of IR and several clinical trials were designed to alter IR prior to or concurrent with interferon / ribavirin therapy and has shown that the outcome is influenced by IR. Besides the Jak/STAT pathway, IFN response is also induced by IRS-1-PI3K/Akt pathway. The molecular basis of non-response is not understood in the absence of cellular or animal models. Furthermore, HCV infection induces autophagic response which might play an important role in viral replication and pathogenesis.

Aim: Our goals are: i) to demonstrate that in chronically infected liver cell model HCV induces insulin resistance (HCV-IR) and ii) it is directly linked to the autophagic response that may modulate viral replication and/or cell death.

Methods: We have generated a chronically infected liver cell model (Huh 7) that continuously secrets HCV in the media. We examined the status of glucose homeostasis, insulin signaling and autophagic response in this cell line by glucose uptake, immunochemical analysis of key proteins potentially involved in the pathways.

Results: Our results show: i) chronic HCV infection caused reduced uptake of glucose, phosphorylation of glucose transporter 2 (Glut 2), up regulation of GSK-3β and phosphorylation of glycogen synthase (GS), all suggesting a disturbance in glucose homeostasis; ii) insulin signaling is disrupted by increased phosphorylation of IRS-1 Ser 312 which is accompanied by a defect in the PI-3K/Akt pathway downstream as indicated by the reduced expression and phosphorylation of Akt at residue 473; iii) Chronic infection is associated with an autophagic response revealed by the over expression of essential marker proteins Beclin 1, Atg 5/Atg 12 and Lamp-1; iv) IRS-1 Ser 312 and Beclin 1 are present in the same immunocomplex suggesting a direct link between IR and autophagic response and v) Beclin 1 is hyperphosphorylated in chronic infection.

Conclusions: i) Chronic HCV infection causes a disturbance in glucose homeostasis and a disruption in insulin signaling pathway, both are suggestive of insulin resistance in a cellular model; ii) HCV-IR is directly linked to an autophagic response which may increase viral load and is possibly induced by a novel phosphorylation based mechanism of Beclin 1; iii) we hypothesize that IFN response may, at least partially, be induced by IRS-1-PI3K/Akt pathway and inhibited in HCV-induced IR by IRS-1 Ser 312 phosphorylation. Our approach to understand the molecular basis of IR and its relationship with IFN response may lead to the development of therapy with more predictive outcome.

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Metagenomic approaches to viral discovery

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Background: Approximately 1.8 million children die from diarrhea annually, and millions more suffer multiple episodes of nonfatal diarrhea. In up to 40% of cases, no causative agent can be identified. Viruses are suspected to cause at least a portion of these cases. Therefore detection of novel or unexpected viruses in diarrhea specimens is the first step in identifying agents that could be responsible for the cases of unknown etiology.

Aims: To use metagenomic methods, which are both systematic and unbiased, to begin defining the spectrum of viruses, including novel viruses, present in stool during episodes of acute diarrhea.

Methods: Pediatric acute diarrhea specimens from cases of unknown etiology in Melbourne, Australia were analyzed using Sanger sequencing for the presence of viral sequences. RNA was extracted from the samples and randomly amplified prior to sequencing. Other PCR based techniques were used to obtain additional viral sequences from the samples and to screen additional cohorts from the US and India for the presence of 'interesting' viruses identified by Sanger sequencing.

Results: In one case, high throughput sequencing revealed the presence of nucleic acid sequences with limited amino acid similarity to viruses in the family *Astroviridae*. The complete genome was sequenced and phylogenetic analysis revealed that the virus, referred to as MLB1, is highly distinct from all previously described astroviruses. Furthermore, the virus was detected in 4/254 pediatric stool specimens from St. Louis, USA and 7/416 from Vellore, India.

Conclusions: A highly divergent novel astrovirus, now referred to as MLB1, was discovered through the application of metagenomic approaches to diarrhea specimens. MLB1 was detected in samples collected on three different continents, suggesting that this virus could be globally wide-spread and of potential importance to human health.

Treatment success with dual proton pump inhibitor plus amoxicillin H. pylori therapy or PPI, amoxicillin, clarithromycin triple therapy

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Background: Studies with CYP2C19 slow metabolizers have shown that the combination of a proton pump inhibitor (PPI) plus amoxicillin (dual therapy) can reliably cure more than 90% of *Helicobacter pylori* infections. Theoretically, the use of a PPI dose that provides equivalent acid suppression with fast metabolizers and slow metabolizers would achieve high cure rates irrespective of the CYP2C19 genotype.

Aim: To evaluate high-dose PPI plus amoxicillin dual therapy for *H. pylori* eradication.

Methods: *H. pylori*-infected individuals (positive by 2 tests) received esomeprazole 40 mg plus amoxicillin 750 mg every 8 h for 14 days. The protocol was planned based on the ''efficient identification strategy'' requiring more than 90% success, with stop criteria of 6 or more failures within 50 patients or a cure rate of less than 80%.

Results: Thirty-six patients (5 women, 31 men), average age 58 years, were enrolled before achieving stop criteria. All were first H. pylori treatments. The intention-to-treat cure was achieved in 26/36 [72.2%; 95% confidence interval (CI) = 56–84%] and in 26/35 per protocol (74.2%; 95% CI = 56–87%). There were no significant side effects. Compliance was 85% or greater in all (100% in 91.6%).

Conclusions: If the hypothesis that consistently high intragastric pH is required to reliably achieve more than 90% H. pylori eradication, our regimen was not sufficient. Success may require more than every 8 h dosing, the concomitant administration of sodium bicarbonate, or the use of a long-acting PPI. However, the result was positive in that dual therapy with the doses tested here was at least as successful as empiric triple therapy.

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A novel small molecule inhibitor of protein kinase D blocks pancreatic cancer growth both *in vitro* and *in vivo*

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Background: Protein kinase D (PKD) is a new family of serine/threonine kinases comprised of PKD1, PKD2, and PKD3 with diverse biological functions including cell proliferation and growth. Pancreatic Cancer (PaCa) is a devastating disease with few therapeutic options. We showed earlier that PKD signaling pathways promote mitogenesis in multiple PaCa cell lines. However, nothing is known about targeting biological functions of PKD in PaCa. Using a high-throughput screening strategy, we discovered a small molecule inhibitor CRT0066101 that specifically blocks activation of PKD family.

Aim: The aim of our study was to determine effects of CRT0066101 in PaCa, both *in vitro* and *in vivo*.

Methods and Results: Our immunohistochemical analysis showed that autophosphorylated PKD1 and PKD2 (activated PKD1/2) are significantly upregulated in PaCa as compared to normal ducts (91% vs 22%; $p<0.001$) and western analyses showed that PKD1/2 are expressed in multiple PaCa cell-lines. Using Panc-1 as a model system, we demonstrated that CRT0066101 reduced BrdU incorporation, increased apoptosis, blocked neurotensin (NT)-induced PKD1/2 activation, reduced NT-induced PKD-mediated Hsp27 phosphorylation (PKD-specific cellular substrate), attenuated PKD1/2-mediated NF-κB activation, and abrogated expression of NF-κB-dependent-dependent proliferative and pro-survival proteins. We showed that CRT0066101 given orally (80 mg/kg/day) for 4 weeks significantly abrogated PaCa growth in a subcutaneous Panc-1 xenograft model ($n=8$; $p<0.01$). Activated PKD1/2 expression in the treated tumor-explants was significantly inhibited with peak tumor concentration (12 μM) of CRT0066101 achieved within 2 h after oral administration. Further, we showed that CRT0066101 given orally (80 mg/kg/day) for 21 days in an orthotopic model potently blocked Panc-1 tumor growth *in vivo* ($n=7$; $p<0.01$). CRT0066101 significantly reduced Ki-67$^+$ proliferation index ($p< 0.01$), increased apoptosis (measured by *in situ* TUNEL assay) of PaCa tumors ($p<0.05$) and abrogated expression of NF-κB-dependent proteins including cyclin D1, survivin, and cIAP-1.

Conclusion: Our results demonstrate *for the first time* that PKD-specific small molecule inhibitor CRT0066101 blocks PaCa growth both *in vitro* and *in vivo* and validates the role of PKD1/2 in PaCa tumorigenesis. We showed that CRT0066101 was orally bioavailable and blocked tumor growth in two distinct PaCa animal models. Thus, PKD is a novel therapeutic target in PaCa.

Can Lipocalin 2/NGAL in exocrine pancreatic secretions distinguish chronic pancreatitis from pancreatic cancer?

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Background: Lipocalin 2 or neutrophil gelatinase associated lipocalin (NGAL) is recently shown as an early biomarker in PC. It is known that secreted NGAL also forms complex with matrix metalloprotease-9 (MMP-9). We hypothesized that expression of either free NGAL or NGAL/MMP-9 complex in secretin-stimulated exocrine pancreatic secretions (SSEPS) will be able to distinguish between normal individuals (NL), chronic pancreatitis (CP), and pancreatic cancer (PC) patients.

Methods: Prospectively, patients with diagnosed CP or PC were enrolled in this study. Asymptomatic patients with no history of pancreatic disease and with at least 1 negative imaging test of the pancreas served as NL group. Every patient underwent upper endoscopy. Following intravenous secretin (16 µg) administration, SSEPS emptied into the duodenum was collected, snap-frozen in liquid nitrogen, and later blindly examined. Free Lipocalin 2/NGAL and NGAL/MMP-9 complex were analyzed by ELISA. Kruskal-Wallis test was used for statistical analysis.

Results: To date, we have enrolled 96 patients (NL=21, CP=23, and PC=52). The level of secreted free NGAL was significantly different between CP/PC and NL with p values of 0.0041 and 0.0035, respectively. Moreover, there was significant difference of NGAL level between CP and PC with p values of 0.0816. The level of NGAL/MMP-9 complex was significantly different between PC and NL with p values of 0.0004, but was not significantly different between CP and PC.

Conclusions: We showed *for the first time* that both free NGAL and NGAL/MMP-9 complex can be effectively measured in SSEPS of NL, CP, and PC. In this trial, free NGAL was helpful in discriminating the presence of pancreatic disease (CP/PC) from NL patients and moreover was able to discriminate CP from PC.

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Neutrophil gelatinase-associated lipocalin inhibits proliferation of human esophageal adenocarcinoma cells both *in vitro* and *in vivo*

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Background: Neutrophil gelatinase-associated lipocalin (NGAL), a member of lipocalin family, is a 25-kDa secreted glycoprotein. NGAL modulates multiple biological functions including cell proliferation, invasion, and metastasis. NGAL is upregulated in multiple gastrointestinal cancers including colorectal, pancreatic, and esophageal adenocarcinoma (EAC). Our group recently showed that NGAL over-expression significantly blocked metastasis of pancreatic adenocarcinoma cells (Cancer Research 68(15): 6100-6108, 2008). However, the biological role of NGAL in EAC is not known.

Aim: To examine the biological role of NGAL in human EAC cells both *in vitro* and *in vivo*.

Methods and Results: We used OE33 (well-differentiated) and Flo-1 (poorly differentiated) EAC cell lines as our model system. We observed that OE33 cells have significantly higher levels of NGAL expression than Flo-1 cells both by qPCR and western blot analyses. To further study the biological role of NGAL in EAC, we created stable NGAL over-expression clones in Flo-1 (Flo1-NGAL) and NGAL under-expression clones in OE33 (OE33-shNGAL) using lentiviral system. We observed that under-expression of NGAL in OE33 significantly increased cell proliferation as determined by clonogenic assays. In contrast, NGAL over-expression in Flo-1 significantly suppressed cell proliferation. We also observed that NGAL under-expression increased cyclin D1 expression, a known cell cycle regulator. This, in part, explained increased EAC cell proliferation secondary to NGAL under-expression. In addition, NGAL over-expression potently suppressed and conversely, NGAL under-expression significantly increased invasion of EAC clones through Matrigel™ *in vitro*. Furthermore, to confirm our *in vitro* findings, we observed that OE33-shNGAL clones strikingly increased tumor volume in a subcutaneouos heterotopic xenograft model in nude mice.

Conclusion: We demonstrated *for the first time* that NGAL under-expression increased cell proliferation and invasion *in vitro*, which could be in part secondary to regulation of cyclin D1 expression. NGAL under-expression also enhanced tumor growth *in vivo.* Thus, modulation of NGAL can be a novel therapeutic target in progression of EAC.

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Inflammatory bowel disease characteristics among Hispanic children in Texas

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Background: Genetic and environmental factors play key roles in the pathogenesis of Inflammatory Bowel Disease (IBD) i.e. Crohn's disease (CD) and ulcerative colitis (UC). Hence, disease variability among different ethnic groups has been noted. However up to this date there is no data about IBD in the pediatric Hispanic population, the fastest growing minority population in the U.S.

Aims and Methods: A retrospective study was conducted to identify patients (pts) ≤ 18 yrs, seen at Texas Children's Hospital and diagnosed with IBD between 1/1/2006 - 12/31/2008. We compared Hispanic **(H)** children with their White **(W)** and African American **(AA)** counterparts in regards to their demographic features, disease characteristics and severity.

Results: There were a total of 190 pts with IBD: 102 **W** (53.7%), 32 **AA** (16.8%) and 27 **H** (14.2%). **H** were comparable to **W** pts with a male predominance (59.3%, 65% respectively), in contrast to **AA** who had a greater female proportion (62.5%, p=NS). The incidence of CD was greater in **W** (61.8%) and **AA** (71.9%) Vs **H** (37%), while UC was more common in **H** (63%) Vs **W** (38.2%) and **AA** (28.1), (p=0.03). The most common presenting symptom in all groups was abdominal pain. None of the **H** pts (0/27) had a family history of IBD while 7.8% of the **W** and 6.3 % of the **AA** had a first degree relative with IBD. The median duration of symptoms prior to presentation was 37 days in **H** vs. 75 in **AA** and 90 in **W**. 44.4% of **H** had frequent limitations of activity (per physician's report), compared to 28.1% in **W** and 60.9% in **AA** (p=0.064). At diagnosis, 90% of **H** received steroid induction therapy (**W**: 66%, **AA**: 65%), 50% were hospitalized (**W**: 32.2%, **AA**: 56.5%), and **H** more often required surgical intervention. (CD; **H**: 20%, **W**: 6.3%, **AA**: 4.3%) (UC; **H**: 5.9%, **W**: 2.6%, **AA**: 0%).

Conclusion: We demonstrate differences between Hispanic patients and other ethnicities with IBD. Hispanic children had more UC than CD and no family history of IBD, both consistent with the adult literature. However CD in Hispanic children was more severe as reflected in shorter time period prior to presentation, higher rates of steroid use, hospitalizations and surgeries at diagnosis. Further studies are needed to better define the burden of illness in Hispanic children.

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Physical activity and eating behaviors in obese children with non-alcoholic steato-hepatitis

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Background: Non-Alcoholic Fatty Liver Disease (NAFLD), a co-morbidity of obesity, is considered the most common cause of chronic liver disease in the world, yet it is unclear why some obese children develop NAFLD and others do not.

Aims: To assess dietary habits, physical activity and healthful knowledge in obese children with biopsy-proven NAFLD in comparison with obese and lean children.

Methods: Children with biopsy-proven NASH comprised the (NA) group. Age, sex, and ethnicity matched control groups consisted of obese (OB) and lean (CO) children with no evidence of liver disease. Subjects were administered the School Physical Activity and Nutrition Survey (SPAN).

Results: 50 pts (CO=13, OB=17, NA=20) had a mean age of 11.8 yrs; nearly all subjects were Hispanic with a M: F ratio of 3:1. The mean BMI% was 51% for CO, 98.2% for OB, and 98.1% for NA. OB and NA had higher Triglyceride with decreased HDL when compared with CO ($p<0.01$). HOMA-IR was significantly higher in NA (4.2) when compared with OB (2.6; $p=0.034$) and CO (0.49; $p<0.001$). SPAN analysis demonstrated that even though the OB and NA had a similar BMI% ($p=0.99$), 35.3% of OB believed they were heavier than their classmates vs. 50% of the NA ($p=0.013$). No statistical differences between the 3 groups in regards to eating breakfast or taking a multi-vitamin. NA consumed the least amount of fruits with only 25% having > 1 fruit/day vs. 47.1% in OB and 69.2% in CO ($P=0.042$). The mean for physical activity score was the lowest in NA with 1.3 vs. 2.41 for OB and 2.08 for CO ($P=0.078$). 16.7% of NA with grade 3 steatosis ate pasta > 2 times/day while 0% in the other groups had > 1 pasta serving/day ($P=0.005$). 50% of pts with grade 1 steatosis had ≥ 3 servings of fruits and vegetables/day vs. only 25% and 16.7% in grade 2 and 3 respectively ($P=0.058$). 50% of pts without fibrosis were on 2 sport teams vs. 0% of NA with fibrosis ($P=0.011$).

Conclusion: While obese children with NASH had the same BMI% as obese controls, more children with NASH viewed themselves as more obese than their classmates. Decreased physical activity and unhealthy dietary habits may be responsible for obese individuals developing liver disease and provide insight into prevention and treatment of NAFLD.

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Three-dimensional modeling of colorectal cancer – NHERF1/EBP50 in Caco-2 cysts

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Background: Colorectal cancer (CRC) is the third most deadly type of cancer in the United States. The consensus has been that activating mutations in the Wnt/β-catenin pathway are present in over 90% of CRC. Our previous study using mouse embryonic fibroblasts has shown that NHERF1/EBP50 (Na^+/H^+ Exchanger 3 Regulating Factor 1; Ezrin-radixin-moesin Binding Phosphoprotein 50), an adaptor protein that associates directly with β-catenin and PTEN, exerts a tumor suppressor effect through interaction with these proteins. NHERF1 is mainly at the apical membrane in normal epithelia, and gene deletion in mice induces ultrastructural abnormalities of the intestinal microvilli. Such multi-dimensional feature of intestinal morphology is, however, difficult to model in conventional 2D monolayer cell cultures.

Aims: In this study, we examined NHERF1 localization and the role of NHERF1 in intestinal morphogenesis in a 3D cyst model of Caco-2 cells.

Methods: Caco-2 cells were embedded in 40% v/v matrigel/growth medium. Immunofluorescence staining of the cysts was performed 2-8 days post embedding, with NHERF1, β-catenin, PTEN, phospho-Histone H3-S10 (PHH3, mitosis marker), atypical PKC (aPKC, apical membrane marker). ZO-1 (tight junction marker), E-cadherin (basolateral membrane marker), and Laminin (basement membrane marker) antibodies.

Results: The Caco-2 cysts developed by successive divisions as marked by PHH3 from a non-polarized single cell. At two cell stage, strong concentrations of NHERF1 were noted in a disc at the center of cell-cell interface. NHERF1 then remained tightly associated with this disc until the lumen formed in the mature cyst. NHERF1 depletion by shRNA induced severe disruption of the cyst morphology, with formation of large asymmetrical spheroids lacking cell alignment and the central lumen. Apical-basal polarity as marked by aPKC, ZO-1, and Laminin were lost or inverted; and the normal apical and basolateral distributions of PTEN and β-catenin, respectively, were both shifted into the cytoplasm and nucleus.

Conclusions: The 3D cyst model shows NHERF1 localization closely resembling that in normal human colonic gland. We suggest that this 3D system provides novel and useful opportunities to model CRC *in vitro*. NHERF1 loss that induces massive disruption of epithelial polarity may, thereby, mark an early important step in CRC development.

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Development of a *Himar1* Transposome™ System to define the genetic basis in probiotic mechanisms in *Lactobacillus reuteri*

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Objective: To identify genetic elements in *Lactobacillus reuteri* ATCC PTA 6475 required for producing secreted soluble factors that inhibit TNF production from activated monocytoid cells.

Methods: We are developing a Transposome™ system based on the *Himar1* transposon. *HimarI* transposase was expressed and purified from *Escherichia coli*. Our transposon was constructed with a P_{23}-promoter-driven chloramphenicol resistance gene (CmR) as a selection marker. The transposase and transposon DNA were assembled as a complex *in vitro* in absence of magnesium ions and electroporated into *E. coli*. Colonies resistant to chloramphenicol were tested for the presence of the CmR gene by PCR. Inverse PCR and Southern hybridization confirmed random insertions of P_{23}-CmR transposon into the genomic DNA.

Results and progress: We successfully expressed and purified active *Himar1* transposase in *E. coli*. P_{23}-CmR transposon containing a selective marker was constructed, purified and assembled with the transposase *in vitro* to create the *Himar1* Transposome™. We detected the presence of the marker gene in chloramphenicol-resistant *E. coli* isolates after the Transposome™ was electroporated into the cells. There was no evidence of plasmid DNA, suggesting that the transposon containing the marker had inserted into the chromosome. Random insertions into *E. coli* chromosome were also confirmed by inverse PCR.

Future directions: Efficiency of transposition will be optimized in *E. coli,* then transferred to *L. reuteri.* After confirmation of random insertions by inverse PCR and Southern blotting in *L. reuteri*, we will generate and screen mutant libraries for the inability (loss-of-function) to suppress TNF production in THP1 cells using the proposed high-throughput screening system. Transcriptomes of integrants exhibiting this loss-of-function will be characterized using DNA microarrays.

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Hepatic osteopontin expression is upregulated in experimental rotavirus-induced biliary atresia

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Background: Osteopontin (OPN) is a glycoprotein with diverse functions including promotion of inflammation and fibrogenesis. Expression of OPN mRNA is significantly upregulated in the livers of patients with biliary atresia (BA), which is a progressive, fibrosing hepatobiliary disease and the leading indication for liver transplant in children. OPN is highly expressed in proliferating bile ducts in BA patients, and degree of expression correlates with degree of fibrosis. Osteopontin expression has not been characterized in an established rotavirus-induced BA mouse model to our knowledge, however.

Hypothesis: Hepatobiliary expression of OPN mRNA is upregulated in mice with BA compared with saline-treated controls, and expression of OPN protein is localized to infected or proliferating bile ducts.

Design/Methods: Neonatal Balb/c mouse pups were inoculated intraperitoneally with 1-4 x 10^7 pfu rhesus rotavirus, a dose sufficient to induce BA in 100% of treated mice, and assessed for BA (pale stools, bilirubinuria, visible extrahepatic biliary strictures/dilatations) at 14-16 days post inoculation (dpi). Livers and extrahepatic bile ducts were removed at 4 dpi (when rotavirus infection is active) or at 15-16 dpi (when bile duct proliferation and fibrosis are present) and formalin-fixed; and peroxidase-based immunohistochemical staining was performed for rotavirus, OPN, and cytokeratin 19 (bile duct epithelial marker) on consecutive sections. In a separate experiment, livers and extrahepatic bile ducts were removed en bloc at 14 dpi and snap-frozen for fluorescent immunohistochemistry and RNA extraction for qRT-PCR to quantitate OPN mRNA expression at 14 dpi.

Results: OPN protein was expressed in normal intra- and extrahepatic bile ducts, and in rotavirus-infected and proliferating intrahepatic bile ducts at all time points in rotavirus-inoculated mice and in saline-inoculated controls. OPN mRNA expression was upregulated more than 3-fold ($p<0.05$) in the livers of rotavirus-inoculated mice with BA compared with saline-inoculated control mice.

Conclusions: OPN is consitutively expressed in extrahepatic bile ducts and as well as in normal, rotavirus-infected, and proliferating intrahepatic bile ducts. Hepatobiliary expression of OPN mRNA is significantly upregulated in rotavirus-treated mice; this may be due to increased biliary epithelial mass with biliary proliferation or to upregulation of OPN expression in proliferating epithelium.

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SIV infection alters expression of granzyme B in intestinal CD4$^+$ T cells of rhesus macaques

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Background: Early pathogenesis of HIV infection is marked by a depletion of intestinal memory CD4 T cells and a concurrent breach of the intestinal epithelial barrier that allows resident bacteria, or their products, to cross the intestinal mucosa. Eventually, intestinal microbial products spill into the circulation, activating peripheral leukocytes. This chronic activation of the immune system provides a means by which HIV can spread throughout the body. This process continues until the immune system is exhausted and opportunistic infections take hold; the hallmark of AIDS. The exact mechanism by which the intestinal mucosa is compromised is not fully understood. Our hypothesis, based on our work with activated human memory CD4 T cells, is that HIV induces CD4 T cells (either as a result of infection or activation) to release Granzyme B (GrzB). We speculate that the interaction of HIV with intestinal CD4 T cells results in the release of GrzB that subsequently causes either; 1) destruction of the extracellular matrix of the intestinal epithelium, or 2) cleavage of tight junction proteins between epithelial cells. Either of these outcomes could compromise intestinal barrier function.

Aims: To assess GrzB expression in intestinal CD4 T cells in rhesus macaques (both healthy and SIV-infected).

Methods: Colon sections were obtained from four rhesus macaques (one healthy and three SIV infected between 6-12 months). An immunohistochemistry double staining protocol was developed specifically for this aim. Ten to fifteen non-overlapping images were taken in the lamina propria (LP). The numbers of $CD4^+GrzB^-$, $CD4^-GrzB^+$, and $CD4^+GrzB^+$ cells were counted and data assessed as percent positive cells per unit area. Data was analyzed by ANOVA and differences between the means were determined with Bonferroni corrected multiple t-Tests. Alpha was set at 0.05.

Results: As expected, chronic SIV infection resulted in significant depletion of CD4 T cells in the LP. However, quite unexpectedly, the uninfected animal possessed the most $CD4^+GrzB^+$ cells (~11%) when compared to the SIV infected animals (<3%).

Conclusions: To our knowledge this is the first time that $CD4^+GrzB^+$ cells have been detected in the LP of the colon in healthy rhesus macaques. They may represent a novel population of cells whose exact function is as yet unknown. These results support our contention that the breach of the intestinal epithelial barrier observed shortly after SIV infection may be caused by the indiscriminant release of GrzB into the LP by SIV-infected or SIV-activated CD4 T cells. Future studies will focus on this population during acute SIV infection as well as whether or not healthy and HIV-infected humans posses a similar $CD4^+GrzB^+$ population.

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MicroRNAs in *Apc*$^{(min/+)}$ adenomas and normal mouse intestinal epithelium.

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BACKGROUND: microRNAs (miRNAs) are small regulatory RNAs (~22nt) essential for cell proliferation and differentiation in all animal cell types and tissues studied. miRNAs generally repress gene expression by binding the 3'-untranslated region of target mRNAs, blocking translation and/or causing degradation of the mRNA. miRNAs can fine-tune and or completely repress the expression of factors important for cell proliferation and differentiation, and their expression levels are disrupted in tumor tissues. The most common initial gene inactivation in familial and sporadic cases of colorectal cancer (CRC) is the tumor suppressor *adenamatous polyposi* (*Apc*). APC is an essential factor inhibiting the proliferative signal of β-catenin (β-cat). In normal intestinal epithelium, APC is highly expressed in the mature, nonproliferative cells along the villi, and is reduced in the proliferative region of the crypt, where β-cat is active. The inactivation of the *Apc* gene in human CRC is modeled in *Apc*$^{(min/+)}$ mice, which spontaneously develop small intestinal adenomas.

PURPOSE: We hypothesize that the miRNAs in the adenomas are dysregulated compared to normal intestinal epithelium, and that some of the miRNAs upregulated and downregulated in the adenomas will overlap with miRNAs enriched in normal crypts and villi, respectively.

METHODS: Total RNA was extracted from adenomas and from scrapings of normal appearing epithelium of C57Bl/6 *Apc*$^{(min/+)}$ mice (n=3), and from scrapings of intestinal epithelium of wildtype (wt) littermate controls (n=2); and from wt mouse villus- and crypt-enriched epithelial fractions (n=3). Small RNAs (<200nt) were hybridized to microarray chips with probes for known miRNA and microconserved elements (MCEs), which may represent novel miRNAs found in progenitor cells. Microarray expression was validated for select miRNAs by quantitative RTPCR.

RESULTS: Forty-one miRNAs displayed a 1.5-fold or greater upregulation and 39 showed downregulation in the *Apc*$^{(min/+)}$ adenoma when compared to normal epithelium from the *Apc*$^{(min/+)}$ mice and wt littermates ($p<0.05$). A smaller number of miRNAs showed differential expression in the crypt vs. villus: 7 miRNA were upregulated in the crypt- and 2 were upregulated in the villus-enriched fractions. miR-152 and -689 showed increased expression in the *Apc*$^{(min/+)}$ adenomas and the crypts; and miR-150 displayed decreased expression in the adenomas was upregulated in the villi. Additionally, 7 of the MCE-MIRs were upregulated in the *Apc*$^{(min/+)}$ adenomas.

CONCLUSIONS: The miRNA enriched in villus and normal control intestinal tissue are attractive candidates for repressing expression of proliferative factors, whereas the miRNA and MCEs enriched in crypt and adenoma tissue might inhibit translation of factors needed to exit the cell cycle or to differentiate and mature. Identification of the mRNA targets of these miRNAs could provide insights into the regulation of proliferation, differentiation and the early development of CRC in the intestine. Furthermore, miRNA are attractive candidates for small molecule drugs. This work could help lead to the development of miRNA-based therapies to inhibit tumor cell growth in CRC.

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Disruption of intracellular calcium homeostasis by an endoplasmic reticulum-localized viroporin domain in the rotavirus enterotoxin NSP4

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Background: As a ubiquitous secondary messenger, the concentration of calcium within cells is tightly regulated and critical for normal cellular functions. Disruption of calcium homeostasis by pathogens is a common strategy used to establish an environment ideal for replication and is associated with pathogenesis. Elevation of intracellular calcium is required for rotavirus replication and caused by the dysregulation of calcium homeostasis at both the endoplasmic reticulum (ER) and plasma membrane (PM) through the actions of nonstructural protein 4 (NSP4). NSP4 increases free cytosolic calcium by increasing both the release of ER calcium stores and calcium entry. While several reports have detailed the NSP4-induced changes in cellular calcium handling, the mechanism(s) of action used by NSP4 remained unknown. Through sequence analysis of NSP4 we identified a 45 amino acid domain reminiscent of viroporins, virally encoded pore-forming proteins. Thus, we hypothesized that **NSP4 disrupts cellular calcium homeostasis by forming pores in the ER and/or PM and mutation of the putative NSP4 viroporin domain will block NSP4-induced changes in intracellular calcium.**

Aims: To determine if the putative NSP4 viroporin domain is responsible for the elevation of intracellular calcium and identify specific mutations that disrupt this activity.

Methods: Mutational analysis was performed to identify amino acids within the putative NSP4 viroporin domain critical for viroporin activity using both prokaryotic and eukaryotic expression systems. Wild-type or mutant NSP4 was expressed in *E. coli* and NSP4-mediated cell lysis was measure to evaluate viroporin activity. Mutants deficient in viroporin activity in *E. coli* were expressed as EGFP-fusion proteins in mammalian cells. Intracellular calcium was measured using Indo-1 and flow cytometry and ER localization was determined by confocal microscopy.

Results: Wild-type NSP4 from multiple strains promoted rapid lysis (20 min) upon induction of expression. Mutation of 3 clustered lysines to glutamic acid or alanine, but not to histidine, blocked NSP4-mediated cell lysis and transmembrane insertion, indicating the cluster of positively-charged residues is involved in membrane insertion of the viroporin domain. Expression of a conserved amphipathic α-helix was sufficient for viroporin activity and disruption of this motif inhibited both NSP4-mediated cell lysis and cytotoxicity in *E. coli*.

In human embryonic kidney 293T cells, expression of wild-type NSP4 caused a 3.7-fold increase in intracellular calcium, but the viroporin activity-deficient mutants failed to elevate intracellular calcium but maintained low intracellular calcium levels similar to cells expressing EGFP alone. Late in rotavirus infection, NSP4 exits from the ER and forms vesicular punctate structures that colocalize with replication complexes called viroplasms. Wild-type NSP4 spontaneously formed punctate structures, which were inhibited by buffering intracellular calcium with BAPTA; however, the viroporin mutants did not spontaneously form puncta but these structures were induced by thapsigargin treatment, a known ER calcium agonist.

Conclusions: NSP4 viroporin activity provides a potential mechanism for the release of ER calcium. Further, viroporin activity and calcium induced puncta formation are separate functions of NSP4. Since ER calcium release is crucial for RV replication, NSP4 is a candidate for antiviral drug development, which may be a treatment alternative for RV-infected immunocompromised children. Further investigation of this function should provide mechanistic insights into NSP4-mediated changes in calcium homeostasis and its effects on rotavirus replication.

Loss of β2-Spectrin may lead to the activation of Telomerase Reverse Transcriptase (TERT) and Hepatocellular Cancer formation

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Background and Aims: Telomerase is a specialized reverse transcriptase (TERT) that synthesizes repeat DNA sequences at telomeres- the specialized ends of chromosomes is suppressed in normal cells by multiple proteins that include TGF-β and Smad3 resulting in the progressive shortening of telomeres with each cell division, ultimately leading to chromosomal instability and cellular replicative senescence or growth arrest. Emerging evidence indicates that β2-spectrin (β2SP), a Smad3/Smad4 adaptor protein required for TGF-β signaling, is a powerful tumor suppressor. β2SP+/-, β2SP+/-/Smad3+/- and β2SP+/-/Smad4+/- mice dramatically develop Hepatic cancers. Because TERT is markedly activated in β2SP+/-/Smad3+/- hepatocellular cancer tissues compared to Smad3-/- tissues, we hypothesized that β2SP interaction could be involved in the interaction with Smad3 to suppress TERT expression in normal tissues, and the loss of β2SP may lead to telomerase gene activation resulting in HCC.

Results: We tested human TERT (hTERT) expression levels in several HCC cell lines that have different levels of β2SP. hTERT expression levels negatively correlate with β2SP by Western Blot analysis. Loss of β2SP in β2SP+/- and β2SP-/- mouse embryonic fibroblasts (MEFs) significantly activated mTERT expression. Co-transfection of a β2SP expression plasmid and hTERT promoter-luciferase construct significantly inhibited the hTERT promoter in β2SP-deficient SNU-398 cells. Depletion of Smad3 by in vitro RNAi and the expression of hTERT-luciferase in Smad3 depleted cells upon co-transfection with Smad3 and/or β2SP expression plasmids suggest that both Smad3 and β2SP are required for the suppression of hTERT. Furthermore, chromatin immunoprecipitation (ChIP) assay suggests binding of Smad3/β2SP protein to hTERT promoter.

Conclusions: Taken together our data suggest that divergent pathways converge on β2SP and Smad3 for the regulation of TERT. Activation of the TGF-β signaling pathway with suppression of TERT provides a strong strategy for generating targeted therapeutics to these lethal human cancers.

An Imageable Retinoid Acid Derivative to Detect Human Cancer Xenografts and Study Therapeutic Dosing to Reduce its Toxicity

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Background: Retinoids are natural or synthetic vitamin A derivatives that regulate a multitude of biological processes in mammalian cells, including metabolism, development, cell proliferation, differentiation, and carcinogenesis. In addition, retinoic acid can reverse oral premalignancy, significantly decrease second primary tumors, and provide a treatment benefit against smoking-related second primary tumors in the head and neck, lung, esophagus, colon, and bladder. This benefit lasts up to 3 years after the completion of treatment but disappears with longer follow-up. These data demonstrate the persistence of retinoic acid preventative effects and also suggest that cancers cells can uptake retinoids. Therefore, retinoids are potential tumor-imaging agents.

Aims: To develop near-infrared (NIR)-labeled retinoid agents to detect human cancers, visualize drug redistribution within the body, determine the optimal biological dose, and reduce any systemic toxicity.

Methods: A synthetic retinoid was labeled with reporters. Binding was tested in human cancer cells and xenograft models. The intracellular trafficking and distribution of the synthetic retinoid was imaged using confocal microscopy. Animals were imaged with the Kodak In Vivo Multispectral System FX, Siemens MicroCAT II SPECT/CT and Inveon PET. Data were analyzed by ANOVA or GLM.

Results: The retinoid agent, but not the free dye, binds to the human tumor cells and is internalized. The retinoid agent can be used to image multiple human cancer xenografts. The signal intensity was initially localized to the mouse liver after the injection but gradually re-distributed from the liver to the tumor. Increasing the injection dose from 0.03 to 0.125 mg did not significantly increase the tumor-to-muscle ratio, but the higher retinoid dose significantly reduced the gain in bodyweight observed in untreated control and low-dose treated animals.

Conclusions: Synthetic NIR-labeled retinoid agents can be used to detect multiple human cancer xenografts as the agent is internalized by cancer cells. The binding of the agent to the tumor xenografts is dependent on the redistribution of the agent. Therapeutic agents labeled with reporters could provide insight for the development of better diagnostic and treatment strategies.

Inhibition of extracellular signal regulated protein kinase ERK by pyridopyrimidine derivative BPIPP

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Background: We have recently identified a novel class of inhibitors of cyclic nucleotide synthesis in intestinal epithelial cells which were effective *in vivo* in a model of acute toxin-induced diarrhea (Kots et al., PNAS, 2008, 105, 8440). A pyridopyrimidine derivative, 5-(3-bromophenyl)-1,3-dimethyl-5,11-dihydro-1*H*-indeno [2′,1′:5,6]pyrido[2,3-d]pyrimidine-2,4,6-trione (BPIPP) suppresses synthesis of cyclic AMP and cyclic GMP via an unidentified mechanism. BPIPP is presumed to have a number of advantageous pharmacological properties including low systemic toxicity and poor bioavailability and is expected to act locally on epithelial cells. Intestinal secretion can be regulated by various endogenous hormones including epidermal growth factor (EGF). EGF stimulates protein kinases of the MAP kinase cascade and is involved in barrier function, restitution, and fluid and electrolyte transport in epithelial cells. Also, MAP kinases are regulated by cyclic AMP and cyclic GMP. The main components of signal amplification in MAP kinase cascade downstream of EGF receptor are GTP-binding protein Ras and protein kinases Raf, MEK, and ERK.

Aims: To evaluate whether BPIPP can influence EGF-mediated activation of MAP kinases.

Methods: Human colorectal carcinoma T84 cells were grown in DMEM/F12 medium and serum starved overnight. Cells were treated with 50 μM BPIPP or 0.1% vehicle (dimethylsulfoxide) in phosphate buffer saline or serum-free medium for 20 min or 24 h, respectively. Levels of phosphoproteins were determined by Western blot with specific antibodies. Cell growth was evaluated with trypan blue, MTT test, and CyQuant DNA-specific stain (Molecular Probes). Ras activation assay (Cytoskeleton) and in vitro MAP kinase reconstitution (Upstate) were performed according to manufacturer's instructions.

Results: EGF (100 ng/ml) induced rapid stimulation of ERK phosphorylation in intact T84 cells. Pretreatment of the cells with 50 μM BPIPP significantly suppressed basal and EGF-dependent activation of ERK but had no effect on other MAP kinases (p38 and c-Jun N-terminal protein kinase). Treatment with BPIPP had no influence on activation of EGF receptor by EGF in intact cells or in membrane preparations. Moreover, BPIPP suppressed ERK activation induced by platelet derived growth factor treatment thus ruling out EGF receptor as a direct target for the drug. BPIPP did not suppress activation of Ras and did not suppress binding of activated Ras to Ras-binding domain of protein kinase Raf. MAP kinase cascade was reconstituted *in vitro* in a system containing purified active truncated Raf-1 and inactive MEK1 and ERK. BPIPP had no effect on Raf activity and on phosphorylation of MEK and ERK unlike known inhibitors of Raf (sorafenib, GW5074, staurosporin) and MEK (U0126). However, activation of Raf by EGF was suppressed in cells pretreated with BPIPP according to confocal microscopy with anti-B-Raf specific antibodies and *in situ* immunoprecipitation assay of Raf activity. This was associated with inhibition of phosphorylation of Raf-1 at regulatory Ser338. Long-term treatment with BPIPP lowered EGF-mediated increase in c-myc expression (4 h) and growth of T84 cells measured by MTT test (IC50 4.9±1.2 μM) and by DNA contents. BPIPP had negligible cytotoxicity according to trypan blue staining or lactate dehydrogenase release (<10%).

Conclusions: BPIPP suppresses phosphorylation of ERK in intact T84 cells by inhibiting activation of upstream protein kinase Raf presumably via interfering with phosphorylation of Ser338 or Raf-1. This effect of BPIPP leads to suppression of cell growth mediated by inhibition of c-myc expression.

β2 spectrin mediates asymmetric division and differentiation of intestine epithelial cells by regulating spindle polarization

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Background: The adult intestinal epithelium of the gastrointestinal tract has a well-defined organizational structure. The epithelium can be divided into two regions, a functional region that houses differentiated cells (villi) and a proliferative region (crypts of Lieberkühn) that represents the epithelial stem cell niche. In the small intestine, finger-like projections protrude from the floor of the intestine, forming villi. Multiple proliferative crypts surround each villus base. All epithelial cells that populate the villus originate from these crypts. Multipotent epithelial stem cells reside in the crypts and give rise to four principal epithelial lineages: absorptive enterocytes, mucin secreting-goblet cells, peptide hormone secreting enteroendocrine cells, and Paneth cells. Three of these cell lineages differentiate as they migrate up and out of the crypts onto the adjacent villus. The fourth lineage, Paneth cells, differentiates with a downward migration to reside at the base of the crypt. It is believed that these different cell types are generated by asymmetric cell divisions of stem/progenitor cells. However, the molecular mechanisms of how stem cells differentiate into four different cell types are only partially understood.

Aim: in the current study, we evaluated the role of β2SP (β2 spectrin) in the differentiation and asymmetric division of stem/progenitor cells in normal intestine epithelium.

Results: Our preliminary data showed that the number of secreting-goblet cells and peptide hormone secreting enteroendocrine cells was reduced in $\beta2SP^{+/-}$ mice compared to the wt mice. The number of cells undergo asymmetric division was also 3 folds lower in $\beta2SP^{+/-}$ mice than in wt mice. In *in vitro* studies, polarization of Par3 and PKCζ was impaired in $\beta2SP^{-/-}$ MEF cells. The coimmunoprecipitation assay demonstrated that β2SP directly bound to Par3 and actin in normal intestine epithelial cells, indicating that β2SP was involved in the spindle orientation and positioning during the asymmetric division of intestine epithelial cells.

Conclusion: β2SP interacts with Par3 and regulates spindle orientation and polarization in the process of cell division, thereby contributing to the differentiation and asymmetric division of intestinal stem/progenitor cells.

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TGF-β signaling mediated suppression of *stat3* transcription in Hepatocellular Cancer

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Background: At least 40% of hepatocellular cancers (HCC) are clonal, suggesting that HCC may develop from malignant transformation of liver progenitor/stem cells. Several signaling pathways, including IL-6/STAT3 and TGF-β are known to be involved in stem cell renewal, differentiation and survival, and are commonly deregulated in HCC. We have previously demonstrated that human and mouse HCC tissues with aberrant TGF-β signaling show increased expression of STAT3. Moreover, down-regulation of the IL-6/STAT3 pathway in a TGF-β disrupted mouse model (β2SP+/-) by crossing with itih4−/− (Inter-alpha-trypsin inhibitor-heavy chain-4) mice resulted in a significant decrease in the incidence of HCC. Treatment of TGF-β unresponsive HCC cell lines with a STAT3-specific inhibitor, NSC 74859, also markedly suppressed growth (Lin, 2009).

Aims: This led us to hypothesize that the TGF-β signaling pathway is a strong candidate pathway for the transition from progenitor to differentiated cells and its disruption may activate the stem cell renewal IL-6/STAT3 pathway.

Methods and Results: First, using early passage MEFs from wild type and β2SP-/- embryos, we demonstrate a 4-fold increase in STAT3 mRNA by RT-PCR. Overexpression or innhibition of β2SP in HCC cell lines results in STAT3 supression or induction, respectively.. We demonstrated that β2SP and Smad3 are bound to the STAT3 promoter only following TGF-β stimulation by ChIP assay. To gain more insights, by mutational analysis, we demonstrated two transcription factor binding sites within the STAT3 promoter, the cAMP-responsive element (CRE) and STAT3 binding (SBE) sites, are essential for TGF-β-mediated regulation of the STAT3 transcription. Subsequent, electrophoresis mobility shift assays (EMSA) demonstrate that the CRE-binding protein ATF-2, Smad3, and β2SP proteins are major components of the TGF-β-mediated STAT3 transcriptional suppressor complex.

Conclusion: These experiments demonstrate a clear link between the TGF-β and IL-6/STAT3 signaling pathways. TGF-β suppression of STAT3 transcription is mediated by a complex including β2SP, Smad3 and ATF-2 at the CRE site of the STAT3 promoter. Inactivation of TGF-β signaling via disruption of β2SP decreases STAT3 suppression and suggests a potential mechanism for malignant transformation in TGF-β deficient progenitor/stem cells. STAT3 may also present an important target for new therapeutics in transformed cancer progenitor/stem cells.

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Probiotic *Lactobacillus reuteri* strains reduce inflammation in neonatal necrotizing enterocolitis in rats

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Background: Necrotizing enterocolitis (NEC) is an inflammatory disease that occurs predominantly in the preterm infant. A delay in intestinal colonization by commensal, nonpathogenic bacteria and colonization with potentially pathogenic organisms often occurs in preterm infants due to a unique environmental exposure, including artificial enteral feeding, treatment with broad-spectrum antibiotics, and the introduction of hospital flora. In addition, the preterm infant has aberrant immune responses which favor a proinflammatory state. Probiotic *Lactobacillus reuteri (L. reuteri)* is known to produce a broad-spectrum antimicrobial substance, reuterin, during anaerobic growth. *L. reuteri* also secretes lactic acid, which decreases the intestinal pH to a level that is unfavorable to most pathogenic bacteria. Finally, *L. reuteri* may modulate immune responses. Recent studies indicated that the immunomodulation by *L. reuteri* is strain-dependent in bacterial lipopolysaccharide (LPS)–activated monocytoid and neonatal pig jejunal epithelial cells. The impact of *L. reuteri* strains in the prevention of NEC and its effects on local cytokine responses have not been investigated.

Aims: To examine the effects of human-derived *L. reuteri* strains on the incidence and severity of NEC and on the inflammatory response in intestine in a neonatal NEC model.

Methods: One-day-old newborn rat pups weighing 5-6 g were separated from their mothers and housed in an incubator. NEC was induced in rat pups by formula feeding and exposure to 10 min of hypoxia (5% oxygen, 95 % of nitrogen x 3 times daily) for 3 days. Some rat pups were fed with formula containing *L. reuteri* strain DSM 17938 or ATCC PTA 4659 (10^6 cfu/g.bw/day). Formula consisted of 15 g Similac 60/40 in 75 ml of Esbilac canine milk replacement. The ileal tissues were evaluated for NEC scores. Cytokines were assessed by MSD multispot cytokine array.

Results: Feeding *L. reuteri* DSM 17938 (n=38) or ATCC PTA 4659 (n=36) in neonatal rats with NEC significantly increased pup survival rate compared to the NEC group without probiotic feeding (n=46). The incidence and severity of NEC were significantly decreased with administration of either strain 17938 or 4659 ($p < 0.001$). Survival rate was greater with strain 17938 than with strain 4659. Levels of cytokines TNF-α, IL-1β, and IL-13 were all significantly decreased in the intestines of rat pups fed with either *L. reuteri* strain, compared to those with NEC without probiotic feeding. The intestines of rats that were formula-fed without hypoxia also demonstrated mild inflammation, with high levels of proinflammatory cytokines, compared to dam-fed rats. Formula-associated inflammation could be suppressed by strain 17938.

Conclusions: *L. reuteri* strains reduce the incidence and severity of NEC in a neonatal rat model. Even though *L. reuteri* strains have been divided into two subsets, immunosuppressive and immunostimulatory on the basis of *in vitro* studies, each subset has potential therapeutic value in our animal NEC model. These results support the contention that probiotic *L. reuteri* may represent a useful to prevent NEC.

βKlotho confers pro-apoptotic tumor suppressive activity on the FGFR4 tyrosine kinase

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Background: Through local cell-to-cell paracrine mechanisms, canonical signaling by the FGF-heparan sulfate (HS)-FGFR transmembrane tyrosine kinase complex controls embryonic development and cellular and compartmental homeostasis in adult organs. Abnormalities in FGF signaling underlie tissue-specific diseases associated with defective cellular homeostasis including tumors. In organs involved in metabolic homeostasis, transmembrane αKlotho and βKlotho (KLB) direct FGFR signaling to metabolic regulation by facilitation of binding and activation by the endocrine FGF19 subfamily (FGF19, 21, 23). KLB and FGFR4 are coordinately expressed at high levels in mature hepatocytes where genetic ablation of FGFR4 disrupts cholesterol/bile acid and lipid metabolism. Genetic ablation or hyperactivity of FGFR4 in hepatocytes has no direct effect on cellular homeostasis in normal liver. However, ablation of FGFR4 caused an acceleration of DEN-initiated hepatocellular carcinomas. This indicated that resident hepatocyte FGFR4 may have a tumor suppressive effect coincident with its metabolic function, but the underlying mechanism is unknown.

Aims: To investigate the role of KLB and FGFR4 partnership in growth/tumor suppression concurrent with bile acid/cholesterol and lipid metabolism.

Methods: We induced the expression of one of the factors at a dose-dependent fashion in both hepatic and non-hepatic cells expressing the other factor constitutively. Assays for population growth, apoptosis flow cytometry, anchorage-independent colony formation, nanoLC-M/MS, Q-PCR, receptor binding and kinase activation were employed. Hepatoma samples were used to investigate the role of KLB in directing the FGFR4 tyrosine kinase to suppression of hepatoma.

Results: We found that the inducible expression of FGFR4 causes rapid dose-dependent induction of apoptotic cell death, and depression of cell population growth and anchorage independent colony formation in response to FGF19 or FGF1 in tumor cells in which KLB is restored. These effects are dependent on the presence of FGFR kinase activity and intact transmembrane KLB. KLB binds and confers high affinity for the FGF19 on the canonic FGF-HS-FGFR complex, and modifies downstream signaling endpoints. The growth and metabolic regulators AKT and mTOR exhibit dose-dependent deactivation in response to the integration of KLB and FGF19, which parallels the dose-dependent appearance of apoptosis, indicating a control mechanism of homeostasis between growth, metabolism and apoptosis. Livers express high level of KLB; however, during hepatocarcinogenesis KLB expression is lost or significantly down-regulated, and restoration of both KLB and FGFR4 to $FGFR4^{-/-}$ hepatoma cells induces apoptosis in response to FGF19.

Conclusions: Our results indicate that KLB, which is significantly reduced in human and mouse hepatomas, plays a key role in FGFR4-mediated hepatoma suppression. This provides new insight into how FGF signaling coordinates liver cellular homeostasis with its metabolic functions. The KLB interaction with the HS-FGFR4 tyrosine kinase complex not only serves to confer high affinity for endocrine FGF19, but also plays an FGF-independent role in directing signaling of the FGF-HS-FGFR4 complex for regulation of metabolism and resident tumor suppression. These results together with our unpublished observations suggest that the ratio of KLB to FGFR tyrosine kinases in general may determine the net outcome among cell growth promotion, growth suppression and regulation of metabolic pathways in tissues where KLB is significantly expressed

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TIRAP contributes to Galactosamine/Lipopolysaccharide-induced acute liver injury in mice.

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Background: Galactosamine/lipopolysaccharide (GalN/LPS)-induced acute liver injury is associated with hepatocyte apoptosis mediated via elevation of tumor necrosis factor (TNF)-α, activation of JNK and downregulation of NF-kB activation. LPS effects are mediated via the activation of cell-surface Toll-like receptors (TLRs) expressed in Kupffer cells and hepatocytes, yet individual roles of distinct members of TLR signaling pathways remains to be fully identified. TIRAP (Toll/IL-1 receptor domain containing adaptor protein) is an adaptor protein that mediates intracellular signaling from TLRs 2 and 4 and plays important roles in the induction of cytokine response during inflammation. However, it is unknown if TIRAP-mediated signaling plays any role in the induction of acute liver injury.

Aims: The purpose of this study was to test the **hypothesis** that LPS/GalN-induced acute liver injury is dependent on TIRAP signaling.

Methods: To test this hypothesis, Adult (12-14 weeks) female wild-type (WT) and TIRAP-/- (KO) mice were injected (i.p) with GalN (700 mg/kg) and LPS (100 μg/kg) and liver tissues were harvested after 1-5 hrs. Serum samples were analyzed for alanine transferase (ALT) activity, a well-established marker of liver injury. Total liver homogenates were analyzed by western blotting for phospho-JNK, JNK, phosho-c-Jun, c-Jun, phospho-ERK, ERK, cleaved caspase-3, cleaved poly (ADP-ribose) polymerase-1 (PARP-1), MMP-9 and IkB-α expression. Nuclear extracts were analyzed by western blotting for the expression of early growth response -1 (Egr-1).

Results : GalN/LPS-mediated induction of Egr-1 (0.5) and JNK signaling as determined by phospho-JNK (0.4), phospho-c-Jun (0.7), and c-Jun (0.4) was impaired in the KO mice as compared to WT (1.0) at 1 hr, whereas total JNK, ERK and IkB-α expression was comparable. Correspondingly, MMP-9 expression (0.5) and caspase-dependent apoptotic cell death pathway is significantly impaired in the KO as determined by cleaved caspase-3 (0.5) and cleaved PARP-1 (0.3) at 5 hrs. Indicative of the extent of liver injury, serum ALT levels were elevated in the WT (744.2 U/L), as compared to KO mice (46.3 U/L) at 5 hrs.

Conclusions: Our findings suggest that TIRAP plays a key role in GalN/LPS-mediated acute liver injury in mice. These results uncover a hitherto unrecognized role of TIRAP signaling in the GalN/LPS-mediated acute liver injury via the induction of Egr-1, MMP-9, JNK signaling and activation of caspase-mediated apoptosis with implications for our understanding of intracellular mediators of innate immune response and identification of novel therapeutic targets for the treatment of acute liver injury.

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Clinical Impact of *Clostridium difficile* in a Pediatric Population Diagnosed by Stool PCR

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Background: The epidemiology of *Clostridium difficile* infections (CDI) is changing. Diagnostic methods have become more sensitive with the shift to stool *Clostridium difficile* DNA detection.

Aims: We aim to describe the clinical characteristics of CDI in a single-center pediatric population diagnosed using stool PCR.

Methods: The Texas Children's Hospital microbiology database of stool *Clostridium difficile* PCRs was used to identify patients 0-18 years of age from January 1, 2008 to December 31, 2008.

Results: We identified 253 cases of CDI: 3:2 M:F, 44% White, 40% Hispanic. 20% (n=51) were <1yr of age. For those ≥1yr, 30% had a primary oncological diagnosis. Indication for CDI testing: Diarrhea 67%, abdominal pain 9%, hematochezia 8%. Toxins A and B were both detected in 73% of cases, 24% A only, and 3% B only. Indication for testing, symptoms, and toxin distribution did not vary when comparing immunosuppressed pts with all others. 70% of cases were community acquired (CA). CA cases presented with abdominal pain (34 vs 19%, p=0.03) and hematochezia (13 vs 2%, p=0.02) more often than nosocomial. 34% of CA cases had a prior CDI (p=0.04). Coinciding GI infections were found in 8%. 64% were inpatients at time of diagnosis. 54% of all cases had recent antibiotic use, 21% PPI use, 39% immunosuppressant use. 29% had a prior CDI, of which 37% occurred in oncology pts. Primary use of oral vancomycin was to treat recurrent CDI (87%, p<0.0001), of these cases, only 35% had no CDI recurrence (p=0.04). 70% of pts treated with oral metronidazole as a 1st line agent had no CDI recurrence (p=0.002). CDI complication occurred in 1 case. For pts <1yr, diarrhea (78%) and hematochezia (12%) were the primary indications for CDI testing. 41% (n=21) were treated. A similar percentage of those treated versus not treated (76 vs 79%) had resolution without recurrence.

Conclusion: We describe the clinical characteristics of CDI in children diagnosed by stool PCR and found they paralleled previously published data which had utilized less sensitive methods of detection. *Clostridium difficile* DNA testing appears to be a clinically effective method for CDI detection in children.

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A role for regulatory T cells and TGF-β in intestinal IgA to rotavirus

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Background: Regulatory T cells play an important role in suppression of effector T cell function and inflammation; however is it not yet know whether regulatory T cells also function in immune induction. Subsets of regulatory T cells express the transcription factor FoxP3 and/or the cytokine TGF-β1. TGF-β1 also plays an essential role in class switching to IgA, suggesting that regulatory T cell derived TGF-β1 may play a role in the induction of IgA.

Aims: The aim of this work is to determine whether rotavirus infection, which induces a profound intestinal IgA response, induces either TGF-β1 or regulatory T cells and whether they are important for rotavirus specific IgA.

Methods: To determine the levels of regulatory T cells induced by rotavirus infection, single cell suspensions from mesenteric lymph nodes (MLNs) and Peyer's patches (PP) from rotavirus infected or uninfected mice were stained and analyzed for regulatory T cells using flow cytometry.

Results: Following rotavirus infection, the total number of FoxP3+CD25+CD4+ T cells was significantly increased in both the MLNs (5 fold) and PP (2 fold) 2-4 dpi. I next determined whether rotavirus infection also altered TGF-β1 levels. In the MLNs of rotavirus infected mice, there was a significant increase in the total number of TGF-β1+ cells (5 fold) and the total number of TGF-β1+CD4+ cells (5 fold). In the PP of rotavirus infected mice, there was a significant increase in the total number of TGF-β1+ cells (4 fold), total TGF-β1+CD4+ cells (3 fold) at three days post infection (dpi), and total TGF-β1+CD19+ cells (5 fold) at four dpi.

Conclusions: The increased number of regulatory T cells and TGF-β1 expression in PP and MLN during rotavirus infection suggests that regulatory T cells might play an important role in the induction of intestinal IgA through production of the critical switch factor, TGF-β1.

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Comparative taxonomic and platform tools development: Application in the pediatric IBS microbiome study.

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Background/Aims: The intestinal microbiome of pediatric patients with irritable bowel syndrome (IBS) is not well defined. In order to characterize this microbiome, stool samples are collected and analyzed from pediatric subjects, ages 7-12 years, using high throughput 16S rDNA sequencing (Roche 454) and high density microarray (PhyloChip) hybridization. Comparisons of these two platforms present several challenges. While both target 16S rDNA sequences, the outputs for these two methods are very different – OTUs identified by PhyloChip are based on a minimum of 11 probes (25mers) while the 454 method generates 200-500 bp of the 16S rDNA sequences. Moreover, the classifiers used for each method utilize two different taxonomic data sets RDP (454) or Hugenholtz (PhyloChip).

Methods: To perform this comparison, we have developed a pipeline comprised of two distinct methods: traditional RDP Classification and Sequence Mapping (RDPC-SEQMAP) using RDP trained taxonomy. The RDP Classification method utilizes a local copy of RDP Classifier to determine the identity of each sequence utilizing an 80% bootstrap cutoff. The Sequence Mapping using RDP trained taxonomy method begins by creating a taxonomic reference file. This file contains the RDP Classifier taxonomy for each sequence listed in the RDP and Hugenholtz unaligned data sets. After mapping each input sequence against our unaligned data sets we can obtain the mapped sequence's taxonomy via our taxonomic reference file. This produces an identical output as compared to RDP Classifier that can be analyzed using one set of tools. The RDP Classification and Sequence Mapping output data is split into taxonomic bins, analyzed, and normalized for advanced analysis using a built-in R package (princomp) for PCA analysis. The first dataset that we analyzed through the RPDC-SEQMAP pipeline was the Mock community data, which was used as a positive control. Next, we analyzed the 8 pediatric control and IBS samples (blinded) that have been sequenced thus far.

Results: Mock community identification using RDPC-SEQMAP resulted in positive identification of all expected bacteria. Using Hugenholtz taxonomy, pediatric stool samples analyzed via 454 sequencing and PhyloChip shared 17 phyla that represented more than 99% of the data sequenced, while 2 additional phyla were detected by 454 alone. Phyla represented only by the 454 sequencing may correspond to newly described species not covered by probes on the PhyloChip. The PhyloChip described more precisely the taxa present in the certain phyla such as the Proteobacteria that are overrepresented (40% bacterial sequences) compared to what is usually found by 454 sequencing in the fecal microbiome of healthy adults(<5%).

Conclusions: In the future, we will extend this analysis with a larger number of clinical samples (80) and extend this comparison to a deeper level of taxonomy (Class/Order/Family). This will enhance the comparisons of these different platforms and deepen our understanding of their advantages and limitations to monitor the composition of the gut microbiome. We are also planning to make the tools developed for platform comparisons available to the public scientific community.

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SOX9 and b-catenin contribute to Paneth cell differentiation via direct regulation of *MMP7*

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Background: The small intestinal epithelial cells are derived from stem cells within the crypts of Lieberkühn; these stem cells are needed for the regeneration of the proliferative compartment and give rise to transit amplifying cells that differentiate into all intestinal epithelial cell lineages. Proliferation and cell fate decisions are regulated by a number of pathways, namely Wnt, Notch, hedgehog, and TGF-β/ BMP signaling. In the small intestinal epithelium, β-catenin and SOX9 are both highly expressed in the proliferating immature cells, but the expression of β-catenin and SOX9 is also high in the Paneth cell nucleus. We reported that *Sox9* is required for Paneth cell differentiation. In mice that are SOX9 deficient specifically in intestinal epithelial cells, no Paneth cells are formed and the major Paneth cell markers, Lysozyme, *Cryptdin1,* and *6*, *MMP7*, and *EphB3*, are not detected. Others reported that the elevated β-catenin expression is necessary for Paneth cell differentiation. It was reported that a slight decrease of β-catenin gene dosage induced a major defect in Paneth cell differentiation and that acute activation of β-catenin induces *de novo* specification of Paneth cells. In either case, the level of SOX9 expression remains unchanged.

Aims: We hypothesized that some of the Paneth cell specific genes are direct targets of both SOX9 and β-catenin.

Methods: A statistical analysis of microarray data using crypt cell samples collected from *Sox9* mutant and littermate controls identified *MMP7 (*Matrix Metallopeptidase 7) as a possible target of SOX9. We performed *in vitro* reporter assays and demonstrated that both SOX9 and β-catenin transactivate the MMP7 promoter. The binding sequences were confirmed by EMSA and Chromatin immunoprecipitation experiments using mouse intact crypt cells showed significant enrichment in the promoter region both for SOX9 and β-catenin.

Results: SOX9 and β-catenin directly bind to one of the major Paneth cell marker genes *MMP7* promoter in intact intestinal crypt cells and regulate its activity.

Conclusions: SOX9 and b-catenin contribute to Paneth cell differentiation via direct regulation of *MMP7.*

SNP-mediated translational suppression of ER mannosidase I accelerates the onset of end-stage liver disease in alpha1-antitrypsin deficiency. S. Pan*, L.

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The unfolded protein response boosts the efficiency of endoplasmic reticulum (ER)-associated degradation (ERAD) by impairing the proteolytic down-regulation of ER mannosidase I (ERManI) which targets misfolded glycoproteins for proteasomal degradation. Homozygous expression of the misfolded Z variant of alpha1-antitrypsin, which is a risk factor for the development of end-stage liver disease, fails to induce the UPR. As such, the efficiency by which the misfolded Z variant is subjected to ERAD must rely, at least in part, on the low basal levels of ERManI. Considering this potential vulnerability, we asked whether genetic variations in the human ERManI gene might influence the rate at which ZZ patients develop end-stage liver disease. In support of this hypothesis, homozygosity for a single nucleotide polymorphism (SNP) (rs4567(A)) in the 3'UPR of ERManI coincides with an accelerated onset of the end-stage liver disease. Functional studies demonstrated that the SNP generates a conditional hypomorphic allele that suppresses the translation of ERManI in response to ER stress caused by the Z variant, possibly impairing the liver's capacity to deal with the rapid accumulation of misfolded alpha1-antitrypsin. Taken together, the present study demonstrates the utility of functional studies to validate the contribution of a SNP in disease pathogenesis, and introduces a novel paradigm in which a subtle defect in the multilevel regulation of gene expression can modify a classical gain-of-toxic-function disorder. The suspected contribution of microRNAs is currently under investigation.

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The neonatal mouse as a model to study probiotic-host interactions: Implications for acute rotaviral gastroenteritis

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Background: Enteric and diarrheal diseases exact a heavy toll on children in developing countries. In addition to causing nearly two million deaths per year, repeated gastrointestinal (GI) infections during infancy place children at high risk for lasting defecits, including stunting, cognitive impairment, poor school performance, and decreased fitness and productivity. Current treatment and prevention strategies are limited in scope. Oral rehydration solution and zinc therapy attenuate only dehydration, enteric vaccines demonstrate limited efficacy in developing countries, and antibiotics are effective against a limited spectrum of pathogens, with widespread use potentially contributing to the emergence of drug-resistant organisms. As a complementary approach, commensal-derived probiotics, or beneficial microbes, are promising therapeutic agents for acute gastroenteritis. Multiple trials with different probiotic organisms report an average reduction of the duration of diarrhea in children by about one day. However, an understanding of the mechanisms underlying probiosis is currently lacking, preventing the selection of optimum probiotic strains or combinations to manage specific GI infections.

Aims: Using the neonatal mouse as a new model in which to study probiotic-host interactions, we sought to to determine how probiotics reduce the duration of rotavirus diarrhea. We hypothesized that either of two strains of human-derived probiotic *Lactobacillus reuteri*, a naturally-occurring commensal microbe, modulate the mouse microbiome while stimulating both innate and adaptive immunity.

Methods: We administered *L. reuteri* to CD1 mouse pups via daily gastric gavage. We used fluorescence *in situ* hybridization to determine whether *L. reuteri* colonizes mouse pups, and both denaturing high-performance liquid chromatography and 454 next-generation sequencing to assess probiotic changes to the intestinal microbiome. We measured epithelial turnover with BrdU and immunohistochemistry, and we analyzed *ex vivo* organ cultures with multi-analyte liquid bead array flow cytometry to document probiotic-associated changes in antibody and cytokine profiles.

Results: Untreated neonatal mouse pups have yet to be colonized naturally by murine *L. reuteri*, and neither of two human-derived strains colonizes the developing mouse GI tract. During transient passage, *L. reuteri* causes strain-specific temporal changes in GI microbial diversity. Furthermore, treatment with probiotics increases the rate of enterocyte proliferation and migration and enhances production of immunostimulatory cytokines in the distal GI tract.

Conclusions: These results are among the first mechanistic data supporting the role of probiotic therapy in modulating acute gastroenteritis, emphasizing the importance of probiotic strain selection for the treatment of specific enteric and diarrheal diseases in children.

The cell specific contribution of nitric oxide to the pathogenesis of necrotizing enterocolitis

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Background: Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in premature infants. Nitric Oxide (NO) is one of the mediators of NEC. Argininosuccinate lyase (ASL) is the only enzyme in the body capable of generating arginine, the substrate for NO production.

Aims: We hypothesize that the cell specific contribution of NO is important in the pathogenesis of NEC. Our aims are to generate a novel conditional knockout mouse model in which endogenous NO production is impaired at a *cellular level* in different intestinal compartments and to establish a mouse model of necrotizing enterocolitis.

Methods: Using Cre Lox technology, we created mouse models with different tissue specific knockouts (KOs) of ASL in smooth muscles, and macrophages. Newborn pups were allocated either to breast feeding or gavage formula feeding. Pups in each group were subjected to hypoxia and hypothermia two times a day for three days and then sacrificed. Ileum was collected for histology, protein, mRNA measurement.

Results: The tissue specific ASL KOs in smooth muscle and macrophages demonstrate lower levels of ASL by western blotting and mRNA analysis. The macrophage KOs demonstrate lower cytokine response to stimulation with LPS. In the smooth muscle ASL KO, none of the 19 pups in the breast fed group (10 controls and 9 tissue specific KO) developed NEC. Of the 27 pups in the formula fed group, 2 out of 13 pups (15%) in the control group and 4 of 14 pups (28.5%) in the tissue specific KO group showed evidence of NEC (P=0.6). The smooth muscle specific KOs trended towards early mortality compared to the controls (P=0.6).

Conclusions: We generated novel tissue specific conditional knockouts of ASL in which endogenous NO production is reduced in a *cell specific* manner. We have successfully established a mouse model of NEC. Preliminary results with the smooth muscle ASL KO show a trend towards increased mortality and increased incidence of NEC supporting the importance of cell specific contribution of NO to the pathogenesis of NEC.

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Serologic correlate of protection against norovirus-associated gastroenteritis

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Background: Norovirus infection is the leading cause of acute non-bacterial gastroenteritis and in vulnerable populations can lead to severe or even fatal illness. Since human noroviruses have not been successfully grown in tissue culture, and no small animal model exists, human challenge studies must be undertaken to elucidate Norovirus pathogenesis and immune response. Recent studies have shown that human noroviruses bind to specific carbohydrate histoblood group antigens (HBGAs) expressed on the surface of intestinal epithelium, and that expression of these genetically determined antigens determines host susceptibility to infection with Norwalk virus.

Aims: We hypothesized that antibodies that block NV-HBGA binding are associated with protection from clinical illness following NV exposure.

Methods: We developed an HBGA blocking assay to examine the ability of human serum to block the interaction of NV virus-like particles with H type 1 and H type 3 glycans. We analyzed serum samples from human subjects who had been inoculated orally with Norwalk virus. Viral gastroenteritis was defined as vomiting plus one other symptom, or moderate diarrhea alone (>200g watery feces).

Results: There was a high correlation between the H type 1 and H type 3 synthetic glycan assays ($r=0.977$, $p<0.0001$); the H type 1 assay was more sensitive ($p<0.0001$). Among 18 infected secretor-positive individuals, blocking titers peaked by day 28 post-challenge and were higher for individuals who did not develop gastroenteritis than for those who did at days 0, 14, 28, and 180 ($p<0.05$ for each). Additionally, 6 of 6 without gastroenteritis had measurable blocking titers (>25) compared to 2 of 12 with gastroenteritis ($p=0.0015$).

Conclusions: Blocking antibodies correlate with protection against clinical NV gastroenteritis. This knowledge will help guide the evaluation of new vaccine strategies, and elucidation of the nature of immunity to the virus.

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Carbohydrate digestion in Congenital Sucrase-isomaltase Deficient (CSID) and Recurrent Abdominal Pain (RAP) children assessed by ^{13}C-Sucrose and ^{13}C-starch breath tests.

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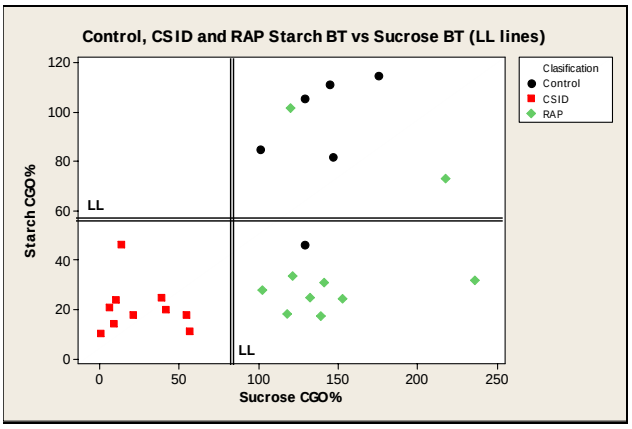
Background: Starches contribute about half of the food energy needs to the child's diet. Malabsorption of sucrose is associated with abdominal pain, bloating and diarrhea. A genetic disorder called Congenital Sucrase-Isomaltase Deficiency (CSID) is suspected when these symptoms follow sugar ingestion and are relieved by a sugar elimination diet. Diagnosis is made upon demonstration of very low or absent sucrase activities in duodenal biopsy enzyme assays with a normal morphology. Treatment is by sucrose elimination diet and oral enzyme supplementation with sacrosidase. Clinical reviews of CSID have suggested that some of these patients also have starch malabsorption. The presence of coexisting starch and sucrose intolerance makes childhood dietary management very difficult and reduces the value of oral sacrosidase supplements. Confirmation of starch intolerance is made difficult because of lack of a specific substrate for mucosal biopsy enzyme assays. **Aim:** To examine starch digestion in a series of children with CSID and a representative population of RAP with normal biopsy disaccharidase activities and similar symptoms. **Methods:** Six control subjects; eleven CSID patients and 10 RAP age matched patients were investigated with oral breath test using 20mg of UL-^{13}C-substrates and periodic $^{13}CO_2$ breath enrichment analyses normalized for % of glucose oxidation (%CGO) as previously reported. UL-^{13}C-substrates (G & ST; 20mg.each, Isotec, Miamisberg, OH) were given in 10gm unlabeled glucose oligomers (10%) vehicle after an overnight fast and reference sampling on 2 separate days. Serial $^{13}CO_2$ breath enrichments (every 15 min x 9) were assayed using a $^{13}CO_2$ infrared spectrophotometer (POCone®, Otsuka Electronics, Tokyo, Japan). A Coefficient of Glucose Oxidation (CGO) was calculated for breath enrichments to adjust for individual differences. Normal control subjects were used to define %CGO lower reference levels (LL) defined as mean -1 SD (75 ± 19 %CGO; LL 56). Because %CGO were found to be relatively constant in the period of 30-90' after load, these values were averaged for each individual. **Results:** All RAP patients had normal ^{13}C-sucrose BT with CGO > 79% and all CSID < this LL (see figure). The normal control group had a LL value for ^{13}C-starch of 56 %CGO. Eight of the RAP patients fell below control LL. Within the RAP group (46 ± 24 %CGO), the LL was of 20%CGO and 1 had < 20%. In the CSID group (19 ± 11 %CGO) 6 patients <20%CGO and 2 patients = 20%). Sucrose digestion was abnormal in CSID (p = 0.000) but normal in RAP patients (p = 0.560). Starch digestion by CSID patients was below the 20% of CGO LL found in RAP patients (p=0.000) but both CSID and RAP populations fell significantly below the normal LL of 56% CGO (p = 0.001 and 0.003 respectively). **Conclusions:** Starch digestion was abnormal in 8 of 10 children with Recurrent Abdominal Pain (RAP) and a combination of non-invasive sucrose and starch ^{13}C-BTs was able to differentiate RAP from CSID, two defects of carbohydrate digestion.



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Extracellular ATP and P2Y2 purinergic receptor-mediated signaling in a mouse model of endotoxin induced acute liver injury

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Background: Extracellular ATP via the activation of P2Y2 purinergic receptors influence multiple hepatic functions. Despite its ubiquitous expression, the role of P2Y2 purinergic receptor activation in the pathogenesis of endotoxin-induced acute liver injury is currently unknown. ATP in the extracellular milieu, released from the injured and infiltrating neutophils and platelets at the sites of injury, is believed to function as a 'danger signal' modulating inflammatory cascades and extent of tissue injury.

Aims: Therefore, the purpose of this study was to test the **hypothesis** that extracellular ATP via the activation of P2Y2 purinergic receptors plays a key role in the induction of endotoxin-induced acute liver failure in mice.

Methods: Adult (10-12 weeks old) male wild type (WT) and P2Y2-/- (KO) mice were injected with saline or LPS (E.coli 0111:B4, 100 μg/kg) + Galactosamine (GalN, 700 mg/kg) intraperitoneally. Liver tissues were harvested at 1, 2 & 5 hrs and serum samples (ALT), total and nuclear protein extracts (Western blots) isolated from liver tissues were analyzed for markers of liver injury.

Results: LPS/GalN injection induced acute liver injury, as evidenced by the elevated serum ALT (12-fold vs saline controls; 5 hrs) in the WT mice with significant attenuation in the induction of serum ALT in the KO (2-fold). Correspondingly, early activation of c-Jun N-terminal kinase (JNK) signaling, induction of matrix metalloprotease-9 and early growth response-1 (Egr-1; master regulator of inflammatory cytokines) at 1 hr, as well as the induction of apoptosis and hepatocellular injury, as evidenced by the elevated expression of cleaved caspase-3 and PARP at 5 hrs in the WT livers, were significantly impaired in the KO.

Conclusions: Our findings suggest that P2Y2 purinergic receptor activation is critical for endotoxin-induced acute liver failure in mice. Attenuated apoptosis and distinct impairments in the early induction of pro-apoptotic JNK signaling, Egr-1 and MMP-9 in the KO mice suggests a role for P2Y2 purinergic receptor activation in the induction of apoptotic liver injury, with implications for the development of targeted therapies for acute liver failure.

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Nutritional influences on mucosal epigenetic development and colitis in mice

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Background: Inflammatory Bowel Diseases (IBD) are chronic illnesses that are thought to develop secondary to a pathologic interaction between the immune system and the intestinal microflora that is transmitted by the gut mucosa. Based on epidemiologic and monozygotic twin studies, IBD has been recognized as disorders where developmental epigenetic changes may play an important role. The most stable epigenetic alteration is the methylation of cytosines at CpG dinucleotide sites (DNA methylation). DNA methylation commonly correlates with the transcriptional silencing of associated genes. A methyl-donor diet (MDD) has been specifically found to be effective in inducing permanent changes in DNA methylation in murine models. In this study we addressed whether prenatal and early postnatal exposure to MDD induces prolonged alteration in colonic mucosal DNA methylation and gene expression. Additionally, we assessed if the MDD exposure affects colitis susceptibility.

Aims:
1. To assess the effect of a methyl donor diet during early development on subsequent colitis susceptibility

2. To assess the effect of a methyl donor diet during early development on colonic mucosal DNA methylation followed by validation with bisulfite pyrosequencing

3. To assess the effect of a methyl donor diet during early development on subsequent colonic mucosal gene expression in mice followed by validation with real time RT-PCR

Methods: C57Bl/6J mothers received a MDD or control diet (CD) throughout pregnancy, and through lactation. Offspring of both the MDD and the CD mothers were transitioned to control diet at postnatal day 21 (P21) until P30 or P90. Colonic mucosa was collected for the execution of whole genomic, DNA methylation specific amplification microarrays, as well as gene expression microarrays. Other animals were transiently exposed to dextran sulfate sodium (DSS) in their drinking water to chemically induce colitis at both P30 and P90.

Results: The infant mice reversed from MDD at both P30 and P90 became more ill, lost more weight and had shorter colons by the end of the DSS experiment compared to the control animals ($p<0.01$).

Conclusions: Our preliminary findings show that prenatal and early postnatal exposure to MDD induces increased colitis susceptibility in P30 and P90 mice following even a 69 day reversal to control diet in infant mice. We are currently generating whole genomic DNA methylation and gene expression microarrays with colonic mucosal DNA and RNA from the experimental animals.

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Norwalk virus nonstructural protein p22 induces Golgi disruption with dependence on a mimic of an endoplasmic reticulum export signal

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Background: Protein trafficking between the endoplasmic reticulum (ER) and Golgi apparatus is central to cellular homeostasis. ER export signals are utilized by a subset of proteins to rapidly exit the ER by direct uptake into COPII vesicles for transport to the Golgi.

Aims: We sought to determine the function of p22 during Norwalk virus (NV) replication and/or pathogenesis.

Methods: We transfected NV genomic RNA or p22 alone from a cDNA plasmid to determine the cellular effect(s) of p22 upon susceptible cells.

Results: The Norwalk virus nonstructural protein p22 contains a YXΦESDG motif that is well-conserved throughout human noroviruses and mimics a di-acidic ER export signal in both sequence and function. However, unlike normal ER export signals, the ER export signal mimic of p22 is used to ultimately inhibit normal COPII vesicle trafficking, which then leads to Golgi disruption and inhibition of Golgi-dependent cellular protein secretion, and this is the first reported function for p22. Disruption of the Golgi apparatus was also observed in cells replicating Norwalk virus, which may contribute to pathogenesis by interfering with cellular processes that are dependent on an intact secretory pathway.

Conclusions: These results indicate that the ER export signal mimic is critical to the pathogenic function of p22, shown herein to be a novel antagonist of ER/Golgi trafficking. This unique and well-conserved human norovirus motif is therefore an appealing target for antiviral drug development.

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Loss of the TGF-*β* Smad3/4 adaptor protein *β2SP* results in G_2-M phase arrest and delayed liver regeneration following partial hepatectomy

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Background: Liver regeneration following partial hepatectomy occurs primarily through normally quiescent hepatocytes that exit G_0, re-enter the cell cycle, and undergo one or two rounds of replication, with restoration of liver mass and function. We already demonstrated the key tumor suppressor role of the Smad3/4 adaptor protein β-2 spectrin (β2SP) and its loss is associated with activation of hepatic progenitor cells following partial hepatectomy.

Aims: These observations led us to further pursue the role of β2SP in normal hepatocyte proliferation.

Methods and Results: We subjected wild type and $\beta2SP^{+/-}$ mice to 2/3 partial hepatectomy and sacrificed them at serial time points afterward.Analysis of cell proliferation by pRb, PCNA, and p-Histone immunohistochemistry, however, demonstrated a significant seven-fold decrease in p-Histone labeling in $\beta2SP^{+/-}$ mice at 48 hours post-hepatectomy. Analysis of cell cycle proteins by western blot confirmed a delay in G_2/M phase transition, with a three-fold decrease in expression of cyclin A and cyclin B1 and a four-fold increase in p-Cdk1 in $\beta2SP^{+/-}$ mice compared to wild type at this time point. By 72 hours post-hepatectomy, however, there was no significant difference in nuclear p-Histone labeling or p-Cdk1 expression and both groups returned to baseline proliferation state by 7 days post-hepatectomy. Further analysis of cell cycle inhibitors demonstrated a significant four-fold increase in p53 expression and two hundred-fold increase in p21 expression in $\beta2SP^{+/-}$ mice beginning at 24 hours and returning to baseline by 72 hours post-hepatectomy. In addition, we demonstrate elevated expression of phospho-p53 (ser15) and phospho-p53 (ser20) with an associated decrease in p-STAT3 expression in $\beta2SP^{+/-}$ mice, suggesting activation of the DNA damage pathway, G_2-M phase arrest, and delayed liver regeneration.

Conclusions: Here, we report that β2SP plays a key role in hepatocyte proliferation and its loss results in a delay in G_2/M phase transition. We believe that β2SP may play a key structural role and its loss results in increased susceptibility to DNA damage and dysregulated cell cycle. Our findings may contribute to a greater understanding of the role of β2SP as a tumor suppressor and contribute to our knowledge of tumor biology and hepatocyte proliferation following hepatectomy or transplantation.

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Galectin-3 Induces Human Pancreatic Stellate Cell Activation and IL-8 Production via NF-kB signaling

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Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly desmosplastic tumor with an innate resistance to therapy. Human pancreatic stellate cells (HPSCs) have been shown to produce the abundant extracellular matrix (ECM) associated with PDAC, and this stroma may contribute to malignancy. Galectin-3, a member of a family of β-galactoside–specific lectins, is localized in intracellular and extracellular milieu and plays multiple biological functions. Recently, gal3 has been found to be involved in tissue fibrosis in the liver, lung and colon. However, the role of gal3 in pancreatic tumor fibrosis is unknown. In the present study, we determined the functional role of gal3 in pancreatic stellate activation and cytokine production.

Methods: Recombinant gal3 (rgal3) was prepared and used to evaluate the role of extracellular gal3 on the activation of HPSC. Genetic alterations of gal3 levels using a lentivirus system were used to evaluate intracellular functions of gal3 in HPSC. HPSC proliferation and migration were assessed by the MTS and Matrigel migration assays. IL-8 production was determined by ELISA. Transcriptional activities of NF-κB and IL-8 were determined by reporter assays. An IKB mutant construct (IKBM), Mutagenesis of the IL-8 promoter at the NF-κB site, and an inhibitor of NF-κB (Bay11-7082) were used to determine the signaling pathways evoked by gal3 in HPSC cells.

Results: HPSC cells expressed gal3 constitutively and rgal3 stimulated HPSC proliferation and migration. Conditioned medium from MPanc96 cells in which gal3 was down-regulated had a reduced effect on the growth of HPSC cells compared with medium from vector controls. Rgal3 dramatically induced production of IL-8 from HPSC in a dose dependent manner. Gal3 also stimulated NF-κB transcriptional activity, and the NF-κB inhibitor Bay11-7082 inhibited IL-8 transcriptional activity and its induction by gal3. Furthermore, co-transfection of the NF-κB mutant construct IKBM and the IL-8 promoter construct (-1481) completely blocked the induction of IL-8 activity by gal3. After mutation of the NF-κB site in the IL-8 promoter, rgal3 lost its ability to induce IL-8 transcriptional activity. Up-regulation of gal3 by lentivirus gal3 cDNA in HPSC increased phosphorylation of ERK and cyclinD1 expression, while down-regulation of gal3 by lentivirus gal3 shRNA in HPSC decreased phosphorylation of ERK and cyclinD1 expression.

Conclusion: Collectively, these results suggest that gal3 activates HPSC cells and induces IL-8 production via NF-κB signaling. Gal3 may therefore play a critical role in promoting the dense fibrosis associated with pancreatic cancers.

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Novel inhibitors of Norwalk viral protease

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Background:

Noroviruses, such as Norwalk virus, are an important human pathogen, being responsible for the vast majority of nonbacterial gastroenteritis. The health and economic burdens of this disease are enormous. To date, there are no therapeutics or vaccines for this disease and the only treatment physicians have is rehydration. There is therefore an urgent need to develop countermeasures to prevent and/or control the viral infections.

Aims:

The Specific Aims of this research are to rationally design and synthesize first potent inhibitors of Norwalk viral protease and to solve the crystal structures of the protease-inhibitor complexes in order to develop inhibitors with improved activity.

Methods:

Noroviral protease (NV^{Pro}) is a good drug target, as its function is essential for viral replication. The crystal structure of NV^{Pro} suggests that the protease is a chymotrypsin-like protease with the residues Cys139, His30 and Glu54 as the catalytic triad, we will design a series of peptidomimetic compounds having an electrophilic group, such as an aldehyde or an α, β-unsaturated ester. The hypothesis is that these compounds should be potent NV^{Pro} inhibitors with their electrophilic groups covalently bound to the sidechain of the residue Cys139.

Results:

1. Based on the similarities between NV^{Pro} and rhinovirus 3C protease with many known inhibitors, we have designed and synthesized a small series of peptidomimetic compounds, among which several novel inhibitors have been obtained with K_is as low as 500 nM.
2. Aldehyde is a good electrophilic group for protease inhibition, while α, β-unsaturated ester seems not effective.
3. Our best inhibitor, SYC-010, has been co-crystallized with recombinant Norwalk viral protease, which was diffracted to 3.2 Å. Preliminary results indicated the presence of the inhibitor at the active site. We are now optimizing the co-crystallization conditions in order to obtain an improved resolution.

Conclusions:

Rational, structure based design has led to the discovery of first inhibitors of Norwalk virus protease.

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Folate derivatives contribute to TNF suppression by *Lactobacillus reuteri* through modulation of MAPK pathways

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Background: Select strains of probiotic *Lactobacillus reuteri* produce low molecular weight (LMW) factors (<3kDa) that can inhibit production of the pro-inflammatory cytokine, TNF, from activated human myeloid cells. Potential immunomodulins include a folate derivate and long-chain folylpolyglutamates. We identified LMW factors produced by *L. reuteri* ATCC PTA 6475 and investigated the mechanism for probiotic-mediated TNF suppression.

Aims: To identify low molecular weight secreted factors from *Lactobacillus reuteri* that contribute to TNF suppression and to understand the mechanism responsible for probiotic-mediated TNF inhibition in human monocytoid cells.

Methods: Candidate immunomodulins were identified by mass spectroscopy, and a targeted mutagenesis strategy was adopted to explore their mechanism of action. TNF cytokine production was measured by sandwich ELISA. RNA was isolated from a human myeloid cell line (THP-1) activated with a Toll-like receptor (TLR) 2 agonist in the presence of LMW factors from either wild type or mutant *L. reuteri* ATCC PTA 6475. Gene expression studies were performed with a whole genome gene expression platform (Illumina HT12) as well as real-time PCR arrays (SABiosciences). Spotted transcription factor macroarrays (Combo Protein/DNA Array) were used to study transcriptional regulation.

Results: *L. reuteri* ATCC PTA 6475 produces a folate compound and long-chain folylpolyglutamates, which decrease TNF secretion from activated myeloid cells. An insertion mutation in *L. reuteri folylpolyglutamate synthase 1 (fpgs1)*, an enzyme responsible for adding the polyglutamate tail to folate, causes a loss in folylpolyglutamate production and diminished ability to inhibit TNF. Wildtype *L. reuteri* downregulates expression of TNF mRNA while the *fpgs1* mutant does not, indicating that regulation of TNF by these LMW factors occurs at the transcriptional level. *L. reuteri* conditioned media prevents activation of several MAPK transcription factors, including AP-1 and CREB. Gene expression analysis revealed that wildtype *L. reuteri* downregulates TAB1 (mitogen-activated protein kinase kinase kinase 7 interacting protein 1), a protein necessary for TAK1 autophosphorylation and downstream activation of MAPK pathways JNK and p38α. Downregulation of TAB1 is not seen with the *L. reuteri fpgs1* mutant.

Conclusions: *L. reuteri* produces a folate derivative and long-chain folylpolyglutamates that diminish TNF production by inhibiting MAPK-regulated transcription factors and preventing expression of TNF mRNA and protein. An insertion mutation in one of the *L. reuteri* genes responsible for synthesizing folylpolyglutamates results in diminished folylpolyglutamate production and reduced ability to inhibit TNF.

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P2X7 purinergic receptor activation is necessary for endotoxin-induced acute liver injury in mice

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Background: Endotoxin-induced acute liver injury is initiated via the activation of inflammatory cascades in kupffer cells with eventual induction of hepatocellular apoptosis and necrosis which culminates in acute liver failure. P2X7 purinergic receptors are ligand gated ion channels expressed at the cell surface of monocytes, macrophages and hepatocytes. Extracellular ATP-mediated activation of P2X7 receptors plays key roles in the induction and maturation of pro-inflammatory cytokines. However, the role of P2X7 receptor activation as a potential mediator of endotoxin-induced acute liver injury is currently unknown.

Aims: The purpose of the study was to test the **hypothesis** that extra cellular ATP-mediated activation of P2X7 purinergic receptors plays a key role in the induction of endotoxin-induced acute liver injury.

Methods: Adult (12-14 weeks) wild type (WT) male and P2X7-/-(KO) mice were injected (i.p.) with galactosamine (GalN, 700mg/kg) and lipopolysaccharide (LPS, 100μg/kg) or saline, a well-established experimental model for the study of acute liver failure in mice. Liver tissues were harvested at 1, 2 and 5 hours after injection and serum analyzed for alanine transaminase (ALT) activity, a well established marker of liver injury. Total liver homogenates were analyzed by western blotting for the activation of JNK, ERK and NF-kB signaling pathways.

Results: After 5 hrs of GalN/LPS treatment, serum ALT was elevated 17-fold in the WT as compared to saline controls with significant attenuation of liver injury in the KO mice (5-fold). Correspondingly, GalN/LPS induced robust activation of JNK, ERK and NF-kB signaling in the WT within 1 hr of treatment with attenuated induction in the KO mice.

Conclusions: Our findings suggest that P2X7 plays an essential role in endotoxin-mediated acute liver injury in mice, potentially via the early induction of JNK, ERK and NF-kB signaling, critical for the induction of pro-inflammatory cytokine expression and progression of hepatocellular injury. These results highlight a hitherto unrecognized role of P2X7 receptors in the pathogenesis of acute liver failure with implications for the development of targeted therapies

Imaging Liver Cancer in Mice with Multi-Target-Specific Agents

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Background: Liver cancer is the fifth most common cancer caused deaths worldwide and its incidence is increasing. For diagnosis of liver cancer 50% of patients require invasive biopsies. In fact, histological confirmation of liver cancer from a limited sample remains a complex undertaking, and is often impossible. Furthermore, the cellular heterogeneity of the disease reduces the accuracy of diagnostic assays. Recent developments in molecular imaging provide the technology to study disease noninvasively and permit the analysis of disease status at the level of the entire body or individual lesion, as well as at the cellular level. These approaches can be used to study different components of a disease in whole body to provide a comprehensive disease picture and enhance the diagnose accuracy.

Aims: To use multiple target-specific molecular imaging agents and multiple imaging modalities to study liver cancer both in vitro and in vivo.

Methods: Two target-specific MMP and RGD peptides imaging agents were synthesized for this study. Imaging studies were performed in cancer cells and xenograft models. Cell study was carried out by confocal microscopy. In vivo imaging studies (mice (n=10)) were performed with peptide imaging agents and ^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) using optical, CT and PET scanning. Image data were analyzed using ANOVAs or general linear models.

Results: The MMP peptide imaging agent, but not the free dye, bound to liver tumor cells *in vitro* and was internalized into the cells. Both cell and animal data confirmed that MMP imaging agent is taken up by liver cancer cells. RGD imaging agent was used to display blood vessel formation in the tumor regions. ^{18}F-FDG PET imaging exhibited glucose metabolic stage in the disease site. It is found that the molecular imaging agents had different distributions in the body and differentially internalized into liver cancer cells. All target-specific agents yielded high tumor-to-background ratios after injection.

Conclusions: Target-specific molecular imaging agents can be used to study liver cancer *in vitro* and *in vivo*. Noninvasive imaging with multi-target-specific molecular imaging agents could provide a tool for simultaneously studying multiple liver disease components.

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Dietary history and risk of advanced hepatic fibrosis in veterans with chronic hepatitis C infection

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Background: Approximately 30% of individuals with chronic hepatitis C infection (HCV) develop advanced liver disease. Little is known about the association between customary diet and HCV-related liver disease risk. Veterans using the VA have a high HCV burden. Representing <2.7% of the population, they are estimated to comprise 11% of the 4.1+ million HCV cases in the U.S.

Aims: Our aims were: 1) to evaluate multiple dietary factors, including novel factors like daily copper intake, as potential risk/protective factors for advanced HCV-related liver disease in male veterans, and 2) to compare dietary intake in our sample to published guidelines for adult males.

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Methods: We performed a nutritional epidemiology case-control study in HCV-infected male veterans with: a previous clinical liver biopsy performed within last 3 years and seen at a VA Hepatitis C Clinic; and without: HIV, Hepatitis B, decompensated liver disease or cancer. Interviewers administered a risk factor survey and the Block Food Frequency Questionnaire. Dietary intake in three sets of cases with advanced liver disease (grade A2-A3, stage F3-F4, and steatosis S2-S3) was compared to that in mild disease controls using logistic regression adjusting for smoking, alcohol, age, ethnicity, BMI, total calories, and lag-time between biopsy and survey.

Results: We recruited 91 male veterans. The strongest decreased risk were with lower dietary intake of copper or fiber and risk of advanced fibrosis ($OR_{lowest\ intake\ copper}$=0.07, p-value=0.02, $OR_{moderate\ intake\ copper}$=0.14, p-value=0.02; and $OR_{lowest\ intake\ fiber}$=0.13, p-value=0.02, $OR_{moderate\ intake\ fiber}$=0.17, p-value=0.02). Greatest overall increased risk of advanced liver disease was with lower number daily grain servings and advanced inflammation ($OR_{lowest\ intake\ grains}$=6.58, p-value=0.04, $OR_{moderate\ intake\ grains}$=3.45, p-value=0.11). Daily coffee intake was the only factor universally associated with risk of all sub-types of advanced liver disease (odds ratio (OR) for advanced steatosis, inflammation and fibrosis= 1.66-3.64, though no effects were significant). Daily median intake of several factors including vitamin E, fiber, dairy and fruit was ≥35% lower than US RDA values, while intake of several others including sodium, copper and iron was ≥35% higher.

Conclusions: Our data suggests lower daily dietary intake of several factors may be associated with HCV-related advanced liver disease risk. It also suggests HCV-infected male veterans may benefit from nutritional counseling. However, our results need confirmation in larger samples.

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Omegaven for parenteral nutrition associated cholestasis: Texas Children's Hospital experience

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Background: Parenteral nutrition associated cholestasis (PNAC) can lead to liver failure and death. Ω-3 fatty acids have been shown to have anti-inflammatory properties and cause decreased PN-induced hepatosteatosis in animals. Replacing Intralipid®, Ω-6, with Omegaven®, Ω-3, is hypothesized to decrease severity of PNAC.

Aims: Determine if infections and mortality are significantly lower with Omegaven® compared to Intralipid®. Evaluate for adequate growth in the Omegaven® group.

Methods: Patients with PNAC (conjugated bilirubin, CB>2mg/dL) 14 to 180 days of life, receiving ≥20% of kcal by IV entered this prospective trial to receive Omegaven® 1 gm/kg/d. Controls were obtained by retrospective review of infants with PNAC from 1/06-4/07 at Texas Children's Hospital. Growth was evaluated using post-menstrual age (PMA) specific growth charts. N=26 for Omegaven® and n= 66 for controls.

Results: The mean PMA at birth were 29.4 wks for Omegaven® and 31.3 for controls (P=0.15). PMA at the start of Omegaven® was 41.5 wks. The peak CB for Omegaven® was 10.2 mg/dL vs. 9.7 mg/dL in controls (P=0.77). The mean CB at the start of Omegaven® was 7.6 mg/dL. After initiation of Omegaven®, the mean number of days to resolution of cholestasis, CB <2mg/dL, was 37 d (n=21). Four infants died prior to resolution and 1 had a persistently elevated CB after an abbreviated course of Omegaven®. No deaths occurred related to PNAC with CB >5 with Omegaven® but 9 occurred in controls (P=0.022). Bacteremia occurred in 5 infants with Omegaven® (n=26) and 41 (n=66) with controls (P=0.0004). The mean weight gain was 18 gm/d while on Omegaven®. Only 3 patients in the Omegaven® group dropped below their starting weight percentile.

Conclusion: Our first 26 infants treated with Omegaven® had less mortality due to PNAC with CB >5 and less bacteremia compared to historical controls. Despite a low rate of lipid infusion, weight gain was acceptable for this patient population. The 3 patients who fell in weight percentiles had significant morbidity leading to increased metabolic/caloric needs.

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Molecular Pathology of Wilson's Disease: Excessive Hepatic Copper Disrupts Nuclear Receptor Function

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Background: Our long-term objective is to determine the effect of copper on the DNA-binding activity of hepatic nuclear receptors and expression of hepatic nuclear receptor target genes in a mouse animal model of Wilson's disease. Wilson's disease is an autosomal recessive disease that results in excessive hepatic copper accumulation due to mutations in the Cu-transporting P-type ATPase, ATP7b, and is associated with a variety of symptoms including steatosis, cholestasis, cirrhosis, and liver failure, as well as neurological dysfunction. Wilson's disease is a chronic and severe liver disorder that is fatal if not treated. Chelation and/or zinc therapy started before the onset of severe liver dysfunction has been shown to manage the symptoms of Wilson's disease. The Wilson's disease animal models (Long Evans Cinnamon (LEC) rat and $Atp7b^{-/-}$ mouse) have decreased expression of genes involved in several metabolic pathways, including bile acid synthesis (cholesterol 7α-hydroxylase (Cyp7a1)), cholesterol synthesis (3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), biliary bile acid (bile salt export pump (Bsep, Abcb11) and glutathione conjugated-compound transport (multidrug resistance-associated protein 2 (Mrp2, Abcc2)). The expression of these genes are regulated by nuclear receptors. The direct effect of copper on nuclear receptor function has been demonstrated in studies with the estrogen receptor (ER). Reconstitution of the ER DNA-binding polypeptide with copper resulted in loss of ER DNA-binding activity. Moreover, the binding of copper was higher for the ER DNA-binding polypeptide than zinc, which is essential for appropriate folding of the zinc-finger DNA binding domain. Taken together, nuclear receptor function may be disrupted in Wilson's disease, which provides the opportunity to determine new mechanisms (molecular, structural, and physiological) in a disease model that is directly related to human disease.

Aims: In the present studies, we focused on copper-mediated changes in farnesoid x receptor (FXR) target gene expression. Our hypothesis is that excess copper inactivates nuclear receptors and disrupts signaling pathways critical for maintaining metabolic homeostasis in the liver.

Methods and Results: Electrophoretic Mobility Shift Assay (EMSA) analysis demonstrated a dose-dependent decrease in binding of FXR to FXR response elements on the BSEP promoter with *in vitro* translated proteins treated with 0.5-20 μM $CuSO_4$, which was recovered upon the addition of 10 μM $ZnSO_4$. Binding of FXR from nuclear extracts from the mouse model for Wilson's disease ($Atp7b^{-/-}$) was also decreased relative to the wild-type (C57BL/6) control animals. However, binding of the Sp1 (Cys_2/His_2, zinc containing) and NFKB (non-zinc containing) transcription factors was not different between the control and *Atp7b*$^{-/-}$ mice. In HepG2 cells treated with 5 μM $CuSO_4$, we found an approximately 80% decrease in BSEP mRNA expression by quantitative real-time PCR analysis, which was partially recovered (to 50% of CDCA-treatment alone) with 40 μM $ZnSO_4$.

Conclusions: Copper impairs the function of FXR binding to DNA response elements on the BSEP promoter, which results in decreased mRNA expression of BSEP; however, zinc ameliorates the negative effect of copper on nuclear receptor function. Future studies will measure whether the mRNA expression of other genes essential to metabolic and detoxification pathways, such as, Cyp7a1 (LRH-1, HNF4a, COUP-TF II, LXR-mediated activation), Abcg5/Abcg8 (LXR-mediated activation), CYP3a4/Cyp3a11 (CAR-mediated activation) are disrupted in the presence of elevated copper concentrations. We anticipate that these studies will reveal molecular mechanisms involved in the disruption of metabolic pathways in conditions such as Wilson's disease.

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Travelers' diarrhea and its effect on the human gastrointestinal microbiota

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Background:
The human body is home to a diverse community of bacteria, a majority of which is found in the gastrointestinal (GI) tract. These microbiota perform functions that host cells cannot, such as metabolism of complex polysaccharides and biosynthesis of vitamins. Alterations in the GI microbiota have been correlated to diseases such as obesity and inflammatory bowel disease. Secretory diarrhea is another example exhibiting alterations of the GI community. Up to 60% of individuals traveling from industrialized countries to developing countries acquire a form of secretory diarrhea know as Travelers' Diarrhea (TD). Enterotoxigenic *Escherichia coli* (ETEC) is the leading cause of bacterial TD. It expresses two toxins, heat labile toxin (LT) and heat stable toxin (ST), which ultimately lead to secretory diarrhea. It is thought that ETEC-TD would cause a dramatic decrease in the GI community, however the specific effect of ETEC and its toxins on the GI microbiota has not been studied.

Aim:
This research focused on determining if ETEC-TD causes a specific shift in the gut microbiota and if ST and LT have a similar effect on the gut microbiota.

Methods:
16S metagenomic sequencing has been used to examine the GI microbiome of three groups of individuals: healthy; ETEC positive TD; and non-ETEC diarrheal controls. The differences in the GI populations between these three groups of samples have been examined to determine if LT and ST have similar effects on the GI community.

Results:
All diarrheal samples showed an increase in bacterial species belonging to the phylum Firmicutes and a decrease in the phylum Bacteroidetes compared to healthy controls. Overall, ETEC samples had increased Proteobacteria and Actinobacteria when compared to non-ETEC diarrheal controls. Additionally, samples producing both toxins had increased Verrucomicrobia and reduced Actinobacteria. These data show how the GI microbiota is altered during ETEC infection.

Conclusions:
The observed microbiota shifts caused by ETEC-TD seem to be specific to ETEC; however, comparisons were made to only one pathogen negative control. Processing of more pathogen negative controls is underway to determine if these results are consistent when compared to a larger number of negative controls. Samples in which a single toxin was produced were less diverse than samples that produced both toxins. Samples don't seem to cluster by toxin, however, increased samples are required to show this conclusively.

Chronic liver injury induced CX3CL1/CX3CR1 production regulates lung intravascular macrophage recruitment and angiogenesis in experimental hepatopulmonary syndrome

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Background: Hepatopulmonary syndrome (HPS) occurs in 15-30% of cirrhotic patients and increases mortality. In experimental HPS after common bile duct ligation (CBDL) but not in thioacetamide (TAA) non-biliary cirrhosis, lung capillary density is increased, monocytes accumulate in the microvasculature and angiogenic factors are activated. However, the mechanisms for these events are unknown. The chemokine fractalkine (CX3CL1) can directly modulate monocyte adhesion and activate angiogenic pathways such as vascular endothelial growth factor (VEGF)-A via its receptor CX3CR1 on monocytes and endothelium during inflammatory angiogenesis.

Aims: To evaluate pulmonary CX3CL1/CX3CR1 alterations after CBDL, and to explore whether CX3CL1/CX3CR1 alterations influence pulmonary angiogenesis, monocytes accumulation and HPS.

Methods: Control, 1, 2 and 3 week CBDL and 2 and 8 week TAA treated Sprague-Dawley rats underwent evaluation. The physiologic features of HPS were evaluated using ABGs and microsphere shunting. Pulmonary CX3CL1/CX3CR1 expression and localization were assessed relative to the development of angiogenesis, CX3CL1 signaling pathway activation, monocyte accumulation and the development of HPS. The effects of a neutralizing antibody to CX3CR1 (anti-CX3CR1 Ab) on angiogenesis and HPS after CBDL were evaluated.

Results: Pulmonary angiogenesis occurs in CBDL animals not in TAA. Circulating soluble CX3CL1 levels and lung expression of CX3CL1 and CX3CR1 in intravascular monocytes and microvascular endothelium were increased in CBDL animals as HPS developed. These events were accompanied by pulmonary angiogenesis, monocyte accumulation, and increased VEGF-A expression and signaling. Anti-CX3CR1 Ab treatment reduced monocyte accumulation, decreased lung angiogenesis and improved HPS. These events were accompanied by inhibition of CX3CL1 signaling pathways and a reduction in VEGF-A expression and signaling.

Conclusions: Circulating CX3CL1 levels and pulmonary CX3CL1/CX3CR1 expression and signaling increase after CBDL and contribute to pulmonary intravascular monocyte accumulation, angiogenesis and the development of experimental HPS.

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Texas Medical Center Digestive Diseases Center
2nd Annual "Frontiers in Digestive Diseases" Symposium
Infection in GI Health and Disease

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