

Esophageal Fibrosis is Increased in Children with EoE

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Background: The only long term known complication of Eosinophilic Esophagitis (EoE) is stricture formation. However, the pathogenesis and natural history of this complication is unknown. Eosinophils (eos) are associated with tissue remodeling and fibrosis in other organ systems. Fibrosis has historically been difficult to assess in the GI tract due to technical limitations of mucosal biopsies.

Hypothesis: Children with EoE have increased esophageal fibrosis compared to children with normal esophageal biopsies.

Aim: To determine if esophageal eosinophilia increases esophageal collagen deposition and fibrosis in EoE.

Materials and Methods: Archived esophageal mucosal biopsies from Children's Hospital Denver Jan-Dec 2006 were screened for esophageal lamina propria. Of 890 esophageal biopsies, 290 (33%) demonstrated >2mm of lamina propria when observed under 40X magnification. A subset of well-defined patients (65) was studied as follows EoE (20), GERD (7), normal (21), and indeterminate esophagitis (16). Biopsies were studied in a blinded fashion recording the number of eos/HPF and fibrosis. Fibrosis was characterized by amount of collagen deposition along the basal lamina with 0=loose individual collagen fibrils lacy pattern, 1=tighter collagen deposition with some individual fibrils and 2=densely packed collagen fibrils. Pathologic findings were then unblinded and correlated with clinical data.

Results: Children with a clinicopathologic diagnosis of EoE have increased fibrosis compared to GERD and normal controls. (Fibrosis score 2 EoE vs GERD $p < 0.001$, EoE vs Normal $p < 0.001$ Fisher's Exact test) Figure 1. Increased fibrosis scores correlated with higher numbers of eos per HPF. Figure 2.

Conclusion: Eosinophilic inflammation is associated with increased collagen deposition.

Figure 1. Fibrosis Scores among all patients

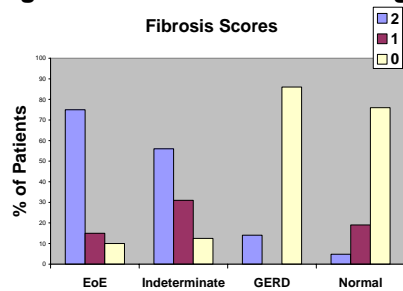
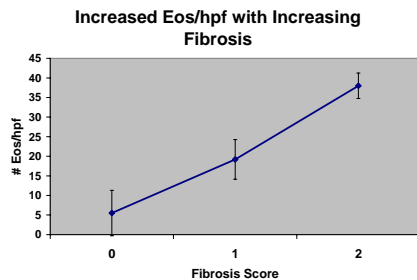


Figure 2. Increased Eos/hpf with increasing Fibrosis among all patients



Effect of Eosinophil Granule Proteins on p27 Expression in Esophageal Epithelial Cells

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INTRODUCTION: p27, a tumor suppressor that regulates the G1 to S phase transition of the cell cycle, is reduced or mislocalized in Barrett's associated adenocarcinoma (BAA). Mislocalization of p27 from the nucleus to cytoplasm disrupts its normal function, and has been associated with poor prognosis in BAA. We have shown that bile acids and hydrochloric acid (HCl) cause mislocalization of p27 from the nucleus to the cytoplasm in normal squamous esophageal epithelial cells (HET-1A) in culture, without affecting total cell p27 levels. Both bile and acid reflux have been associated with an increased risk for development of Barrett's esophagus (BE), but not every patient with BE has a history of GERD. Many patients with symptoms mimicking GERD are later found to have eosinophilic esophagitis (EoE). Eosinophil granule proteins implicated in EoE [major basic protein (MBP) and eosinophil peroxidase (EPO)], have been shown to damage human pneumocytes and guinea pig tracheal epithelium. We sought to determine whether MBP or EPO affects p27 expression in HET-1A cells similar to bile acids and HCl. **METHODS:** HET-1A cells were incubated with MBP at 5-100µg/ml or EPO at 0.8-80µg/ml for 24 hours. Cells were harvested for cytotoxicity studies and immunoblot analysis to determine p27 expression. Indirect immunofluorescence (IF) was performed to determine the subcellular localization of p27. Flow cytometric analysis was used to study the MBP and EPO effects on cell proliferation. **RESULTS:** Exposure of HET-1A cells to MBP or EPO at various concentrations did not affect total cell p27 levels. When HET-1A cells were treated with either MBP or EPO, increased p27 was detected in the cytoplasm, along with reduced but not absent p27 nuclear staining. Flow cytometric profiles were similar for untreated cells and cells treated with MBP or EPO. **CONCLUSIONS:** 1) Exposure of HET-1A cells to MBP or EPO results in increased cytoplasmic p27. 2) The presence of p27 in the cytoplasm may disrupt its normal cell cycle inhibitory function and be an important early marker of dysplasia.

The Effect of the Route of Sensitization in the Induction of a Cow's Milk Allergic Response

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INTRODUCTION: We have previously shown that naturally particulate milk allergens (casein - CAS) are preferentially taken up into the Peyer's patch and induce greater sensitization compared to soluble allergens (α -lactalbumin - ALA) taken up through IECs. However, emerging clinical evidence suggests that other sites such as the skin or the airways may be more likely inductive sites of allergic sensitization to food proteins. As initial antigen exposure is likely to play an important role in food allergy induction, we examined the effect of different routes of exposure using aggregated versus soluble milk proteins on sensitization.

METHODS: C3H/HeJ mice were exposed weekly for six weeks to an aggregated or a soluble cow's milk antigen, CAS or ALA respectively, with the adjuvant cholera toxin. Sensitization routes included oral, cutaneous (skin), intranasal (IN), sublingual (SL), and intraperitoneal (IP). Mice were then challenged orally with the sensitizing antigen at increasing doses, followed by an IP challenge if anaphylaxis was not induced. Anaphylaxis severity was assessed by symptom score and body temperature and antigen specific immunoglobulins in serum (IgE, IgA, IgG1 and IgG2) as determined by ELISA.

RESULTS: All groups of mice exposed to CAS via mucosal routes responded to CAS challenge with anaphylaxis. Percent anaphylaxis was as follows: oral: 87.5%(n=8), skin: 20%(n=5), IN: 100%(n=10), SL: 60%(n=5), IP: 60%(n=5). Mice with more severe symptoms showed a significant decrease in core body temperature: control: 36.3C(Standard error (SE) =0.11), oral: 33.3C(SE=.31), skin: 34.4C(SE=0.76), IN: 33.0C(SE=0.3), SL: 33.2C(SE=0.8), IP: 35.7C(SE=0.11). CAS specific immunoglobulins were elevated at 6 weeks in all groups when compared with controls. Identical experiments using the soluble allergen ALA induced allergic symptoms upon challenge in all groups except controls: oral: 78%(n=9), skin: 80%(n=5), IN: 100%(n=9), SL: 100%(n=5), IP: 80%(n=5). Again, mice with more severe symptoms showed a significant decrease in core body temperature: control: 37.6C(SE=0.24), oral: 36.1C(SE=0.3), skin: 33.9C(SE=0.7), IN: 34.6C(SE=0.3), SL: 34.0C(SE=0.4), IP: 35.5C(SE=0.18).

CONCLUSION: Our data show that allergic sensitization to food proteins can occur through a variety of mucosal routes. In addition, it appears that aggregated food proteins require mucosal sites for sensitization whereas soluble proteins can readily sensitize via the skin. These data suggest that non-oral routes of food allergen exposure may be clinically important in the development of food allergy.

GASTROINTESTINAL ENDOSCOPY SIMULATION TRAINING: WHAT TYPE OF FEEDBACK IS MOST EFFECTIVE?

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BACKGROUND: Feedback has been identified as the most important feature of simulation-based medical education that leads to effective learning. However, the most effective feedback conditions for endoscopy skill acquisition in a simulated setting have yet to be determined.

PURPOSE: This study sought to determine the optimal timing of expert feedback (concurrent versus terminal) in promoting skill acquisition and retention in novices learning to perform colonoscopy in a simulated setting.

METHODS: Thirty novice endoscopists were pre-tested on a bench model colonoscopy simulator task which involved navigating a real colonoscope through a series of marked targets as quickly and accurately as possible. Participants were then randomly assigned to receive feedback either during (concurrent) or after (terminal) each practice trial. All participants underwent 12 trials of practice in their assigned training condition. The effectiveness of training was assessed using an immediate post-test and one week later using both a retention test and a transfer test to a novel path through the simulator. Performance measures included execution time and blinded expert assessment of performance (checklist and global rating scores). In addition, novices were asked to rate the quality of feedback received.

RESULTS: Both groups performed similarly at pre-test ($p > .05$). There was no significant difference in the time to complete the practice session for the concurrent versus terminal feedback group (34.1 min vs. 38.5 min, $p > .05$) and both groups performed similarly during the post-test and retention test ($p > .05$). On transfer testing, however, the terminal feedback group performed significantly better as measured by execution time, checklist and global rating scores ($p < .05$). In addition, performance of the concurrent feedback group decreased significantly on the transfer test as compared with the post-test and retention test ($p < .05$). Students in both groups rated the feedback they received as equally useful, clear and timely ($p > .05$).

CONCLUSIONS: The results of this study show that not all feedback conditions are equally effective. While the performance of participants in both the terminal and concurrent feedback groups improved, the use of terminal feedback resulted in better learning as demonstrated by superior performance on transfer testing. Incorporation of terminal feedback into technical skills training curricula may therefore help to greatly enhance the educational benefits of endoscopy simulation technology, while ensuring effective utilization of faculty time.

Investigator's statement (check all that apply):

This research involves human subjects research.

The IRB approval #: 22179 (University of Toronto IRB)

This research involves the use of laboratory animals. IACUC # is _____.

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Objective: GERD in children results in significant complications and morbidity. Though adult studies have shown an increase in GERD and its sequelae, studies in children are lacking. We aimed to describe the epidemiology of GERD in hospitalized U.S. children.

Methods: We used the Pediatric Hospital Information Survey database encompassing initially 32 and now 46 U.S. children's hospitals. We analyzed clinical and financial data of hospitalized children from 1995-1999 and 2002-2006. ICD-9 codes for esophageal reflux and complications were used for data queries. Collected data included age, gender, race, and discharges/year. Discharge rates were calculated per 10,000 hospital discharges.

Results: The percentage of hospital discharges with a diagnosis of GERD increased from 3.5% of 1,848,349 discharges in 1995-1999 to 4.3% of 2,128,205 discharges in 2002-2006. The rate of discharges with a diagnosis of GERD increased from 1995-1999 and from 2002-2006(table 1). For the 2 time periods, there were 2 and 6 cases of esophageal adenocarcinoma, respectively. From 2002-2006, there were 56 cases of Barrett's esophagus. The rate of funduplications performed increased from 1995-1999, but decreased from 2002-2006(table 1). Total hospital charges for patients with a primary diagnosis of GERD increased yearly from 1995-2006(table 1).

Conclusion: The hospitalization rate for GERD and its sequelae in U.S. children is rising. The total cost of hospitalization is increasing, as are complications of GERD, including esophageal cancer. However, surgical management has decreased. Overall, the burden of GERD and its complications is rising in hospitalized U.S. children.

	1995	1996	1997	1998	1999	p-value	2002	2003	2004	2005	2006	p-value
Hospital discharge rate for children with discharge diagnosis of GERD (per 10,000 discharges)	377.3	448.5	474.9	503.6	543.66	p<0.001	366.8	365.2	413.4	430.6	446.9	p<0.001
Rate of fundoplication for children with primary discharge diagnosis of GERD	26.8	27.3	28.3	29.5	30.5	p<0.001	36.2	31.8	31.3	25.7	23.8	p<0.001
Total adjusted charges (in millions) for children with primary discharge diagnosis of GERD	28.41	30.408	36.086	50.077	54.319	p<0.001	58.963	65.152	80.561	85.523	88.057	p<0.001

TITLE: Severe Painless Gastrointestinal Bleeding: An unusual presentation of Helicobacter pylori associated Duodenal Ulcer in Children of South East Asian Origin.

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ABSTRACT BODY:

Introduction: Children with Helicobacter pylori associated duodenal ulcer usually present with abdominal pain and vomiting. Severe gastrointestinal bleeding due to duodenal ulcer is rare in children. We report three children of South East Asian origin who presented with severe painless gastrointestinal bleeding due to helicobacter pylori related duodenal ulcer.

Case Reports: All three children (Table) were of South East Asian origin. All of them were previously healthy and did not have prior history of abdominal pain or vomiting. They presented acutely with symptoms of gastrointestinal bleeding. The examination in all was remarkable for pallor and signs of hypovolemia resulting in tachycardia and orthostatic hypotension. They required fluid resuscitation for hypovolemic shock and two of them received blood transfusions in the local hospital prior to referral. Endoscopy showed nodular gastritis with large duodenal ulcers and no active bleeding. All of them received combination therapy of two antibiotics and proton pump inhibitor. They all did well.

Conclusion: We report an unusual presentation of helicobacter associated duodenal ulcer in children. This phenotype is characterized by severe gastrointestinal bleeding without any prior gastrointestinal symptoms and is seen in children of South East Asian origin.

Patient	Age (years)	Gender	Race/Nation	Presentation	Hemoglobin at Presentation	Endoscopic Findings	Blood Transfusion
1	11.5	M	Asian/ Vietnamese	Pallor, melena, dizziness	8.1 g/dl	Duodenal ulcer Helicobacter gastritis	Yes, 1 unit
2	14	F	Asian/ Chinese	Pallor, dizziness	8.9g/dl	Two Duodenal ulcers, Helicobacter gastritis	No
3	12	F	Asian/ Korean	Pallor, hematemesis	6g/dl	Gastritis, Duodenal ulcer Helicobacter gastritis	Yes, 2 units

Evaluation of the Relationship between Upper Airway Disease and Gastroesophageal Reflux Disease Using Diagnostic Testing

Context: Brief episodes of gastroesophageal reflux (GER) are part of the normal physiologic maturational changes of the nervous and musculoskeletal systems. Complications of GER are classified as GER disease (GERD). GERD is often suspected of being associated with respiratory or otolaryngology conditions such as laryngomalacia, subglottic stenosis, recurrent pneumonia, asthma, laryngeal edema and acute life threatening events in addition to esophagitis. Unlike adults, pharyngeal regurgitation is more common in infants, placing them at an increased risk of supraesophageal complications.

Objectives: Evaluate the relationship between upper airway disease and GER using diagnostic modalities, including pH probe, scintigraphy, histology and visual assessments. Evaluate the efficacy of pharmacological acid blockade.

Study Design/Setting/Participants: Retrospective chart review at The Children's Hospital of Philadelphia and satellites from January 1, 2004 to December 31, 2007. Inclusion criteria include ages 0 to 18 years, all races and ethnicities, referral for clinical suspicion of GERD, and airway disease. Exclusion criteria include eosinophilic esophagitis, known esophageal motor abnormalities, connective tissue disease, esophageal malignancy, or pregnancy.

Results: (Preliminary data, 17 of 157 subjects evaluated) A Reflux Finding Score (RFS) >8 has a 95% certainty to correlate with laryngopharyngeal reflux. The Cotton Meyer grading scale for subglottic stenosis (SGS) was not statistically associated with the reflux index ($r^2 = 0.02$, $p=0.7$, CI = -3.2 to 2.2). The degree of esophageal inflammation did not statistically correlate with the reflux index, $r^2 = 0.02$, $p=0.7$, CI = -0.3 – 0.2. There was not a statistically significant association between the RFS and an increase in reflux index, $r^2 = 0.2$, $p=0.2$, CI = -1.2 to 0.3.

Conclusions: The degree of SGS by Cotton Meyer or inflammation by histology are not associated with the severity of GER by reflux index. The RFS may not provide a statistically significant grading scale for GERD.

Kristin Fiorino

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Title: Feasibility and Application of 3-Dimensional Ultrasound for Measurement of Gastric Volumes in Healthy Adults and Adolescents

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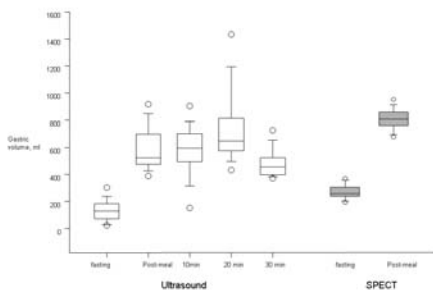
Purpose: Abnormal gastric accommodation to a meal results in dyspepsia. Current methods to measure gastric volume (GV) are invasive or involve ionizing radiation.

The aims of this study were: 1. To compare fasting and postprandial (PP) GVs measured by 99mTc-SPECT and 3-Dimensional Ultrasound (3D-US) in adults; 2. To assess the performance characteristics of 3D-US measurement of GV during fasting and postprandially; 3. To develop normative data of GVs in 24 healthy adolescents.

Design/Methods: The study included two healthy groups: **1.** 11 adults underwent SPECT and 3-D US simultaneously to measure GV; each adult also underwent a second 3-D US within a week from the first study **2.** 24 adolescents (age 13-17 years¹⁸) underwent one 3-D US measurement. Each 3-D US study included fasting, 300 mL Ensure[®] meal, and 0-30 min PP GV measurements. 3-D US, was performed by one operator with a stationary external probe (1.4-5.8 MHz) and a mechanized volume data acquisition.

Results: Adults fasting and PP GVs by 3-D US and SPECT are shown in [figure1]. Mean delta (PP-fasting) GV was 462±134 mL for 3-D US and 530±49 mL for SPECT (p=0.15). There were larger inter-individual COV for GVs by 3-D US (60.3% fasting, 21.3% PP) compared to SPECT (19% fasting, 9.2% PP). Intra-individual COV for two 3-D US measurements were 84% fasting and 44% average PP. Estimated GVs for the adolescent group (median, 25th-75th IQR) were: Fasting 33 (18-53mL), 30 min PP 330 (284-357mL), and delta GV 281 (240-324mL).

Conclusions: 3-D US is a promising method to measure GV accommodation to a meal. Large COVs reflect, in part, the learning stage in development of this promising technique.



Postural Tachycardia Syndrome (POTS) and Functional Gastrointestinal Disorders (FGID): A role for altered electrical activity of the stomach?

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Background: The cause of abdominal pain in patients with orthostatic intolerance is unclear. Children can be divided in sub-groups based on whether upright tilting replicates symptoms. We investigated whether the electrical activity of the stomach also changes with tilt position.

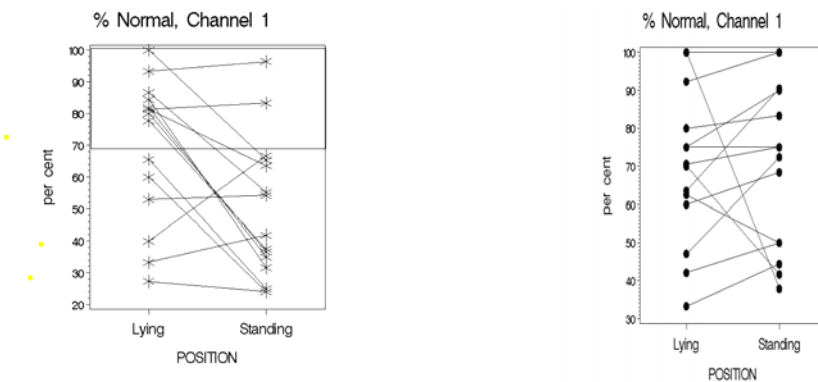
Hypothesis: Children with FGID and POTS have changes in the electrical activity of the stomach during the upright portion of the tilt.

Methods: All children undergoing autonomic testing were enrolled in this IRB approved prospective study. EGG was recorded 10 minutes in supine position and during the upright portion of tilt. EGG findings were correlated with autonomic diagnosis using Wilcoxon Rank Sum test. For the purpose of statistical analysis children were divided into two groups: 1) POTS and or vasodepressor syncope (VDS) 2) Non-POTS group include normal subjects and subjects with autonomic neuropathy.

Results: 30 patients participated (20 females). Mean age 14 ± 3.5 years. 20 had POTS/VDS, 5 autonomic neuropathy, 5 were normal. 11 subjects with POTS replicated symptoms during upright portion of the tilt. When evaluating Channel 1 of EGG, subjects with POTS/VDS, but not those without POTS, showed a tendency for an increase in % arrhythmia ($p=0.02$) and a decrease in % normal gastric activity ($p=0.02$) in the upright position in relation to the supine position.

Conclusion: This exploratory study suggests that the electrical activity of the stomach changes during the upright position in children with POTS/VDS, but not in children without this diagnosis. These changes could reflect abnormal autonomic control of gastric electrical activity and bear some relationship to the chief complaint of pain which worsens in the upright position. Further studies are needed to corroborate these findings.

Legend to Figures Changes in % normal gastric electrical activity lying vs standing. (Stars:POTS subjects; dots: non-POTS subjects) $p=0.02$



Title: The Effect of Erythromycin on the Colonic Motility of Children and Young Adults During Colonic Manometry

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Introduction: Erythromycin, a motilin receptor agonist, is successfully used as a gastro-duodenal prokinetic agent. Its effects on colonic motility have been studied in adults with conflicting results. Given the limited available treatments for colonic dysmotility, further investigation into erythromycin's effects on colonic motility are warranted.

Aims: To study the effect of erythromycin on colonic motility in pediatric patients with recalcitrant chronic constipation/encopresis and other suspected colonic motility disorders.

Methods: Patients referred for colonic manometry were eligible for enrollment. After appropriate bowel cleanout, a colonic manometry catheter was inserted endoscopically. A colonic manometry study was conducted on the next day. Fasting motility was recorded for 1 - 2 hours, then erythromycin lactobionate (EL) 3 mg/kg was administered intravenously, and colonic motility was monitored for 1 – 2 hours following erythromycin. Manometry was then continued per routine, including manometric evaluation after a meal and after intra-colonic administration of Bisacodyl. Colonic pressure tracings were recorded and transferred to a personal computer system (RedTech-GiPC Gastrointestinal System). The Motility Index (MI) (average area under the curve) of pressure tracings at each pressure transducer was calculated for each patient for a period of 15 and 60 minutes before and after EL infusion. Change in MI was compared by Wilcoxon Signed Rank test. A $p < 0.05$ was considered statistically significant.

Results: Twenty patients were enrolled (50% male, mean 10.7 years). The most common indications were constipation with encopresis (60%) and suspected pseudo-obstruction (20%). 70% of patients had normal colonic manometry, and 30% of patients demonstrated a neuropathy. No patients had a true myopathy. Average MI for the 60 minute period prior to and after EL infusion were 254 mm Hg/hr +/- 74 (95% CI 216-293) and 253 mm Hg/hr +/- 94 (95% CI 214 – 291) respectively ($p=0.55$). Average MI for the 15 minute period prior to and after EL infusion were 64 mm Hg/15 min +/- 23 (95% CI 51 – 76) and 69 mm Hg/15 min +/- 32 (95% CI 57 – 82) ($p = 0.45$). On subanalysis, EL did not increase MI in either the normal manometry group or the neuropathy group.

Conclusion: Administration of intravenous EL resulted in no changes in colonic motility index in pediatric patients referred for colonic manometry. Further studies on potential colokinetic agents are warranted in this population of patients.

Respiratory Failure, Compartment Syndrome, Colonic Perforation and Colectomy: Constipation to the Highest Degree

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It is believed that 5% to 20% of the general pediatric population suffer from constipation. The majority of children with constipation are believed to have a functional disorder and are successfully managed on an outpatient basis. However, there are times when functional constipation may lead to significant morbidity and requires immediate intervention. We report three cases of neurologically and cognitively normal adolescents who presented with life threatening fecal impaction. 1) 16 year old male who presented to the Emergency Department with abdominal distension, respiratory distress and bilateral pedal edema. He had an emergent manual disimpaction under general anesthesia. His post-disimpaction recovery was complicated by an ICU stay, foot drop and limp. 2) 15 year old male who presented to the Emergency Department in septic shock with severe abdominal distension and pain. He had an emergent exploratory laparotomy which revealed a megacolon, a perforated cecum, and abdominal compartment syndrome. He had a subtotal colectomy, an ileostomy, and a postoperative period complicated by intraabdominal abscesses, decubitus ulcers, neuropathic lower extremity pain and a temporary tracheostomy. 3) 15 year old male with a long standing history of constipation who presented with a severe impaction and respiratory difficulties and developed cardiopulmonary arrest during an attempt at a clean-out. He required emergent bedside exploratory laparotomy for a perforated colon. These patients were not known to have any underlying disorders predisposing them to severe, life threatening constipation. Hirschsprung's disease was ruled out in all after their acute presentations. They have now all recovered completely. These cases document that although rare, serious complications can occur with constipation. Therefore, it is important to be vigilant in the care of these patients, to educate patients and parents about the dangers of untreated constipation, and to proceed thoughtfully in the management of severe impactions.

A pre-post retrospective study of patients with cystic fibrosis and gastrostomy tubes.
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Background: The impact of inadequate nutrition on the progress of cystic fibrosis (CF) is well documented and gastrostomy tube (GT) feedings appear to be a valuable adjunct in the care of such patients. It is not known whether delivery of GT feedings results in improvements in pulmonary status, an essential factor in increasing lifespan in CF. We conducted a retrospective review utilizing the Minnesota Cystic Fibrosis Center Database. Our hypothesis was that GT feeds would improve weight gain and this improvement would be accompanied by improved pulmonary function.

Methods: Subjects were identified by presence of a GT. Primary outcomes were body mass index (BMI)-percentile, and National Health and Nutrition Examination Survey (NHANES) FEV1-percent-predicted (ppFEV1). For adults ≥ 18 , BMI-percentile was computed using the age of 18. Patients were included if they had at least 5 ppFEV1 observations and at least 1 BMI-percentile calculation before and after GT placement. Patient-wise regression analysis was conducted with modeling of each patient's ppFEV1 measurements separately as a linear function of time with both a step change and slope change. BMI-percent values were compared during four time periods: the two years immediately before GT placement and the first, second, and fourth year after GT placement. The hypotheses were tested using t-tests. The possibility that the changes in lung function may be correlated with the level of lung function at GT placement was tested by regressing the estimated step changes and the estimated slope changes for the 46 patients against their ppFEV1 levels at GT placement and testing whether ppFEV1 at placement was a significant predictor.

Results: 46 CF patients with a GT and at least 5 ppFEV1 observations and at least 1 BMI-percentile calculation before and after GT placement were identified. A total of 3091 ppFEV1 measurements and 3086 pBMI calculations were obtained. The mean estimated step and slope changes in ppFEV1 at GT placement were +0.0861% ($p = 0.9527$) and GT +3.725% per year ($p = 0.0007$). On subgroup analysis, the mean estimated step and slope changes at placement for adults were +1.1138% ($p = 0.5043$) and +7.1593%/yr ($p = 0.0085$) and -0.319% ($p = 0.8691$) and +2.3722% /yr ($p = 0.0278$) for children. The estimated coefficient for the ppFEV1 level at placement was -0.07 (p -value = 0.4291).

For BMI-percentile analysis, 46 patients had at least 1 measurement both in the two years before placement and in the 1st year after GT placement. The change in mean BMI-percentile was +4.2407% ($p = 0.0387$). 39 patients remained a year 2 and 29 at year 4 with mean percentile differences of +13.226%, ($p < 0.0001$) +10.93% ($p = 0.0067$).

Conclusion: Aggressive nutritional management of patients with cystic fibrosis using GT feedings results in significant improvement in their growth. Improvement in growth after GT feedings in this patient population is associated with significant improvement in pulmonary function and this improvement does not appear to depend on the level of lung function at the time of GT placement.

Biomarkers in Children with Non-Alcoholic Steatohepatitis

Wael N. Sayej, MD

Background: Non-Alcoholic Steatohepatitis (NASH) represents a serious form of fatty liver disease, characterized by hepatic steatosis associated with evidence of inflammatory changes and variable degrees of fibrosis. It has been estimated that up to 20% of the population may have fatty liver disease and up to 3% have NASH. Liver biopsy continues to be the main method of diagnosing NASH. This study is aimed at identifying diagnostic and prognostic markers for NASH, which we hope will eventually allow designing a reliable framework for the screening, staging, differential diagnosis and prediction of response to therapy. **Methods:** blood samples were collected from patients undergoing liver biopsies. The blood samples were collected in EDTA tubes and serum was separated, aliquoted, and stored at -80°C for the purpose of this study. Patient demographic data and laboratory test results were collected from their medical records. Enzyme linked immunoassays were performed to check for levels of inflammatory markers that we hypothesized could be potential markers including: Leptin, IL-6, IL-8, TNF, TNFr1, TNFr2, IP-10, IGFBP-1, and TRAIL. Interleukin-1 β and β -NGF were used as negative controls. Statistical analysis (ANOVA, Kruskal-Wallis test, and TTests) was performed using Graphpad Prism software v.5.00. **Results:** A total of 76 patients with blood samples were enrolled in the study, only 62 had liver biopsies. The groups included: NASH BMI \geq 30 (n=19), NASH BMI 25-30 (n=4), NASH BMI<25 (n=3), NASH + Diabetes (n=5), NASH + hepatitis B or C (n=2), hepatitis B (n=8), hepatitis C (n=7), AIH (n=3), hepatitis NOS (n=6), other liver disease (n=4), Obese patients with no liver biopsy (n=8) and Controls (normal liver biopsy or normal weight and LFT's) (n=7). Leptin levels were significantly higher in the NASH and obese patients compared to all other groups, $p < 0.0001$. Levels correlated with the degree of obesity, $p < 0.0001$. However, Leptin did not correlate with the degree of steatosis, inflammation, or fibrosis. Leptin in NASH BMI \geq 30 patients correlated with insulin resistance (IR), $p < 0.0001$. IGFBP-1 levels were lower in NASH patients with IR and obese patients compared to NASH patients with no IR, $p = 0.0386$ and 0.0436 . IP-10 was significantly higher in patients with AIH and hepatitis C infection, $p < 0.0001$ and $p < 0.0342$. TNFr1 levels were higher in NASH patients, $p = 0.0327$. It was also higher in NASH patients compared to obese patients with no liver biopsies, $p = 0.0039$. TNFr2 was also higher in NASH BMI \geq 30 than the other groups, $p = 0.0157$; obese with no biopsies, $p = 0.0128$; and controls, $p = 0.0189$. There was no significant difference in serum levels of IL-6, IL-8, TNF, TRAIL. Interleukin-1 β and β -NGF. **Conclusion:** Leptin is a good marker to measure in the evaluation of NASH. However, it does not give any insight as to the degree of steatosis, inflammation or fibrosis. Obese patients with normal liver enzymes also have higher leptin levels, which might indicate that leptin is only a marker of obesity and not liver disease. TNFr1 and TNFr2 might serve as potential markers for evaluating inflammation in patients with NASH. IGFBP-1 is a good marker to evaluate for insulin resistance in NASH and obese patients. IP-10 is a possible marker to evaluate for acute inflammation thus differentiating AIH or acute infections of the liver from other liver diseases. Further studies are required with larger population numbers to expand on these results and conclusions.

Investigator's statement (check all that apply):

This research involves human subjects research. The IRB approval # is DB#745.

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Long Term Effects of Vitamin Supplementation of HIV-Infected Mothers on Childhood Morbidity and Mortality in Tanzania

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Abstract

Objective: To determine whether maternal multivitamin supplementation has a long-term effect on mortality and morbidity of children up to 5 years of age born to HIV-infected mothers. **Methods:** 1078 HIV-infected pregnant woman were enrolled in a double-blind randomized placebo-controlled trial in Dar es Salaam, Tanzania to examine the effects of daily supplementation with vitamin A, multivitamins or both on maternal and child outcomes. Supplements were provided daily during pregnancy and lactation. Children were evaluated at monthly clinic visits or home visits where information was collected about vital status, diarrhea, cough, fever, respiratory infections, and other common morbidities. **Results:** 776 children were followed through a median age of 51 months. Maternal receipt of multivitamins or vitamin A did not affect child mortality from 6 weeks to 5 years ($p=0.58$ and $p=0.14$, respectively), but maternal vitamin A receipt resulted in higher mortality (RR 1.98, 95%CI: 1.12, 3.51 $p=0.02$) in children from 2 to 5 years of age. Children born to mothers who received multivitamins had a decreased risk of all types of diarrhea (RR 0.80, 95%CI: 0.68, 0.95 $p=0.01$), acute diarrhea (RR 0.85, 95% CI: 0.74, 0.97 $p=0.02$) and watery diarrhea (RR 0.80, 95% CI: 0.68,0.95 $p=0.02$) from 6 weeks to 5 years of age. The reduced risk of watery diarrhea persisted in children from 2-5 years of age (RR 0.70, 95%CI: 0.52, 0.93 $p=0.01$). Children born to mothers who received vitamin A had an increased risk of all types of diarrhea (RR 1.29, 95% CI: 1.03, 1.63: $p=0.03$) and acute diarrhea (RR 1.29 95% CI 1.02, 1.62 $p=0.03$) from 2-5 years of age. **Conclusions:** Multivitamin supplementation to HIV-infected mothers did not affect child mortality but decreased diarrheal incidence up to age 5 years, while vitamin A supplementation increased child mortality and the incidence of diarrheal infections. The effects of maternal micronutrient supplementation during pregnancy and lactation may be observed in children until at least 5 years of age.

Association of Vitamin D Receptor (VDR) in Parkinson Disease. A genetic risk factor for neurodegeneration.

Vitamin D and the vitamin D receptor (VDR) has become an increasingly interesting area of research in a wide variety of disorders. Studies have recognized the relation of VDR gene polymorphisms with prostatic cancer, infectious diseases, type 1 diabetes mellitus, low bone mineral density in post menopausal women, malignant melanoma, chronic periodontitis, renal cell carcinoma, autoimmune hepatitis, breast cancer susceptibility and progression, Grave's disease, celiac disease and IBD. Vitamin D's effects are mediated by its nuclear receptor (VDR). The VDR gene is a large gene spanning over 100kb located on chromosome 12q13, in an area that has been shown to be linked to inflammatory bowel disease. It includes 11 exons and has an extensive 5' promoter region which generates multiple tissue-specific transcripts. Exonic mutations in the VDR gene have significant clinical consequences as is the case with the autosomal recessive disease 1,25-dihydroxyvitamin D₃ dependent rickets type II where any one of several missense mutations lead to an inability to respond to physiologic 1,25-dihydroxyvitamin D₃. However, more subtle variations such as occurs with single nucleotide polymorphisms (SNPs) in the VDR gene occur relatively frequently and have yet unknown clinical effects. This present study focused on a large dataset of Caucasian patients (N=2530 non-Hispanic Caucasian individuals). Our dataset was derived for the purpose genetic studies of Parkinson Disease, however it is a large dataset of Caucasian individuals which are also known to be at higher risk for IBD. We examined 30 tagging SNPs ($r^2 > 0.8$, minor allele frequency > 0.05 in Caucasian) surrounding the VDR gene to capture most of the common genetic variations in the gene. We found several SNPs associated with age-at-onset PD with the strongest association at rs4334089 ($p = 0.0008$). All of them are located in the 5' end of the gene, in introns 1,2 and 4 and immediately to the 5' end of the gene. Our data suggest that VDR polymorphisms are associated with both age at onset and risk for PD in a large family data set. While these results are specific for PD, our methods can be utilized to study any disease process. Given the similarities between the dataset for PD and IBD, these SNPs should be investigated in an IBD dataset.

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Abstract

TITLE: Role of Chenodeoxycholic Acid (CDCA), FXR (Farnesoid X Receptor) and Fibroblast Growth Factor 19 (FGF19) in Parenteral Nutrition Associated Liver Disease

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ABSTRACT BODY: Background: Parenteral nutrition is essential for patients with impaired gut function. Unfortunately, it is associated with Parenteral Nutrition Associated Liver Disease (PNALD; steatosis, disruption in glucose and lipid metabolism, cholestasis, cholelithiasis, cirrhosis and liver failure). During normal enterohepatic circulation, bile acids induce intestinal expression of the newly described metabolic hormone FGF19 via the nuclear receptor FXR. We describe a potential role for FGF19 in metabolic dysfunction during total parenteral nutrition (TPN). Methods: Neonatal piglets, randomly assigned to receive TPN or enteral (EN) feeding for 10 days were implanted with catheters in the jugular vein, carotid artery and stomach. A TPN subgroup received once daily escalating doses of FGF19 intravenously (10mcg–1200mcg) and another oral CDCA (Chenodeoxycholic Acid). The expression of key metabolic target genes was assessed using primers based on conserved genomic sequences. Results: Robust levels of FGF19 were observed in EN portal blood, but FGF19 was absent in the TPN group. The TPN group in comparison to the EN group had significantly lower High Density Lipoprotein (HDL) and higher Low Density Lipoprotein (LDL), triglyceride, cholesterol and Very Low Density Lipoproteins. Total and direct bilirubin was elevated in the TPN group with significantly lower albumin levels. There was significant improvement in bilirubin levels in the CDCA group. Grossly, the TPN livers were pale yellow in contrast to more normal red appearance of EN fed piglets. The TPN group had steatosis with balloon degeneration of hepatocytes and increased glycogen deposits. Atrophic small bowel was evident in the TPN group. CDCA treatment improved gross and histologic appearance of both liver and small bowel. No difference in hepatic expression of FXR, SHP, LXRA or NTCP was noted in either group. BSEP expression was induced in the CDCA Group. The EN group had lower LXRB levels. Preliminary results from FGF19 infused animals showed a significant reduction in LDL, VLDL, triglyceride and cholesterol levels. Conclusion: These results suggest that decreased FGF19 during TPN may contribute to PNALD. CDCA has promising results in gut growth and resolution of PNALD. Partially supported by the American Liver Foundation.

***Tlr2* deficiency protects mice from diet-induced obesity and insulin resistance**

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Introduction: Though toll-like receptor 4 has been implicated in the pathogenesis of the metabolic syndrome, the contribution of toll-like receptor 2 has not been systematically reported. Human gene expression studies show that *Tlr2* is upregulated in adipose tissue and mononuclear cells of obese individuals suggesting that it too may be an important mediator of inflammation that underlies metabolic syndrome.

Hypothesis: The aim of this study was to determine if *Tlr2* deficiency protects mice from insulin resistance, hypercholesterolemia and hepatosteatosis using a model of diet-induced metabolic syndrome.

Methods: Five-to-six week old male C57BL/6 and *Tlr2* deficient mice (*Tlr2*^{-/-}) on the C57BL/6 background were used for all studies. Mice were randomized to five weeks of conventional rodent chow (CHOW) or to a high-fat, high-sucrose (HFHS) diet previously shown to induce key features of metabolic syndrome.

Adiposity was assessed by DEXA scan in a dedicated cohort of mice. Fasting blood samples were obtained at sacrifice for serum insulin, glucose, cholesterol and alanine aminotransferase (ALT). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the HOMA Calculator version 2.2.2 (University of Oxford). Portions of the liver were allocated for routine histology as well as oil-red-o staining and perinephric adipose tissue was collected for gene expression analysis by quantitative real-time PCR (QPCR). Statistical analyses were conducted with two-factor ANOVA and Fisher's post hoc test, significance was set at $P \leq 0.05$.

Results: As expected, five weeks of HFHS feeding was sufficient for induction of obesity in wild type C57BL/6 mice (body fat by DEXA: 28.12% vs. 17.67%, $P < 0.001$). *Tlr2*^{-/-} mice on HFHS were completely protected from diet-induced adiposity (17.72% vs. 28.12%, $P = 0.0009$) and had body fat levels comparable to CHOW fed animals.

Although fasting serum glucose was similar between wild type animals on both diets, it was significantly decreased in *Tlr2*^{-/-} animals on HFHS compared to wild types on HFHS (262.4 mg/dL vs. 333.6 mg/dL, $P = 0.007$).

Greater insulin resistance, as assessed by HOMA-IR, was observed in wild type mice fed HFHS (2.98 vs. 1.27, $P = 0.01$), an effect abrogated in the HFHS group by *Tlr2* deletion (2.1 vs. 2.98, $P = 0.05$). Body weight did not account for the improvements in insulin sensitivity seen in the *Tlr2*^{-/-} group.

Fasting serum cholesterol increased in wild type mice on HFHS (127 mg/dL vs. 173 mg/dL, $P = 0.002$) in accord with previous experiments. *Tlr2* deficiency prevented the increase in serum cholesterol for animals exposed to HFHS (95.8 mg/dL vs. 173 mg/dL, $P < 0.001$), leading to levels comparable to the CHOW group. Similarly, the low density lipoprotein (LDL) fraction rose in wild type mice undergoing HFHS feeding (15.24 mg/dL vs. 26.38 mg/dL, $P = 0.009$) and *Tlr2*^{-/-} mice were protected from the increase (26.38 mg/dL vs. 15.5 mg/dL, $P < 0.001$).

Fasting serum ALT was not different among mice on either diet and histological examination of the liver did not reveal a significant degree of inflammation or necrosis in any group. Consistent with prior observations, hepatic steatosis was present in wild type mice on HFHS (macrovesicular and predominantly lobular) but absent in those fed CHOW. Unexpectedly, *Tlr2*^{-/-} animals on HFHS were completely protected from the development of hepatosteatosis.

Concurrently, HFHS induced an increased expression of *Ccl2* in the perinephric adipose tissue of wild type animals (0.0008 au vs. 0.003 au, $P = 0.001$) which was entirely prevented in *Tlr2*^{-/-} mice (0.0011 au vs. 0.003 au, $P = 0.004$).

Conclusion: *Tlr2* deletion confers dramatic protection against the development of metabolic syndrome. Body fat, insulin resistance and serum cholesterol of *Tlr2*^{-/-} animals on HFHS were all reduced to levels comparable to wild type CHOW fed animals suggesting that intact *Tlr2* signaling is mandatory for development of metabolic syndrome in this model.

The reduced expression of *Ccl2* in perinephric adipose tissue of HFHS *Tlr2*^{-/-} mice may partly explain the improvements in insulin sensitivity observed.

Title: Hepatic fat fraction is associated with abdominal fat distribution and fasting insulin in overweight Latino adolescents

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Background: Non-alcoholic fatty liver disease (NAFLD) is increasing in prevalence in the United States, particularly among Latinos. Insulin resistance and a high proportion of visceral adipose tissue (VAT) both appear to be factors leading to the development of NAFLD, and fasting insulin has been found to correlate with hepatic fat fraction in overweight Latina adolescents. We expanded our previous investigation of these relationships to include overweight Latino boys.

Objective: To assess the relationships between hepatic fat fraction (HFF) and abdominal fat distribution as well as with glucose and insulin values in overweight Latino adolescents, both male and female.

Methods: 52 overweight Latino adolescents, 23 male and 29 female, had abdominal MRI to evaluate abdominal fat distribution and HFF. Blood glucose and insulin were measured at both an outpatient 3-hour oral glucose tolerance test (OGTT) and an inpatient 3-hour frequently sampled IV glucose tolerance test (FSIVGTT). Insulin sensitivity (SI) was estimated from the FSIVGTT using the Bergman minimal model.

Results: HFF ranged from 1.9 to 35.8%. When corrected for age and total body fat mass, HFF correlated positively with visceral adipose tissue (VAT) volume for both boys ($r = 0.57$, $p=0.026$) and girls ($r = 0.44$, $p= 0.026$). HFF was also positively associated with fasting insulin for both genders (boys: $r=0.75$, $p < 0.001$; girls: $r=0.60$, $p < 0.001$), with overall correlation coefficient of 0.65 for both genders ($p < 0.001$). Incremental area under the curve for insulin throughout the OGTT was correlated with HFF for both boys ($r=0.53$, $p=0.043$) and girls ($r= 0.65$, $P < 0.001$), with overall correlation coefficient of 0.51 ($p < 0.001$). HFF was not associated with post-challenge insulin values or SI for either gender. A model including age, gender, VAT, SAT, and fasting insulin explained 60% of the variance in HFF.

Conclusions: Among overweight Latino adolescents, liver fat correlates with visceral fat when controlling for age and total body fat mass. For both genders, fasting insulin is a strong correlate of liver fat, and its predictive utility is not improved by the use of post-challenge measures. Even in the absence of abdominal imaging, fasting insulin may be clinically useful in identifying adolescents of both genders who are at risk for NAFLD.

BILIARY ATRESIA AT THE HOSPITAL FOR SICK CHILDREN: A 33-YEAR EXPERIENCE. O Guttman, C O'Connor, T da Silveira, V Ng, S Ling, E Roberts

Biliary atresia (BA) is a progressive fibro-inflammatory hepatobiliary disease which causes obstruction and obliteration of large, mainly extrahepatic, bile ducts in infants. It remains the leading indication for liver transplantation in children. Apart from persistent conjugated hyperbilirubinemia, clinical presentation of infants with BA is heterogeneous. The aim of this study was to identify both typical features of BA and atypical features in the largest clinical experience with BA in Canada.

Methods: The SickKids biliary atresia database, established since 1987, was reviewed for the period 1973-2006. All patients with additional clinical abnormalities or relevant family history were identified and their charts were reviewed.

Results: Two hundred and thirty-four children with BA were followed at HSC between 1973 and 2006. One hundred and forty (60%) were female. Median age at referral was 61 days. One hundred and ninety-eight patients (86%) underwent Kasai operation at a median age of 69 days (range 6 – 134 days). Liver transplant was performed in 112 patients (47%) at a median age of 12 months (range 5 – 174 months). Among the BA patients treated during this time period, thirty-eight (16%) had associated congenital malformations consistent with aberrant laterality, including situs inversus, polysplenia and cardiac abnormalities. One such patient had a sibling with similar complex laterality defects, but without BA. Other congenital anomalies included genitourinary malformations in 3 patients and musculoskeletal abnormalities in two patients. Cow's milk protein intolerance was identified in 2 patients. Two patients were diagnosed with autism spectrum disorder and 2 others were found to have sensorineural deafness. Hepatocellular carcinoma was identified in 2 patients who subsequently underwent liver transplantation. A number of syndromes were recognized among the BA patients, including Cat Eye, Emmanuel, Rubenstein-Taybi and Trisomy 18. Two patients with first- or second-degree family members with BA were identified, as well as 2 sets of twins discordant for BA.

Conclusions: This is the largest single-centre experience with biliary atresia in Canada. Clinical outcomes are consistent with those reported nationwide. Significant heterogeneity in clinical features of patients with BA is evident in this patient cohort and reinforces the perception of BA as a disorder with multiple pathogenetic mechanisms.

Investigator's statement (check all that apply):

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The chemokine Cxcl10 promotes cholestasis and hepatic inflammation in experimental biliary atresia

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Biliary atresia (BA) is a neonatal disease in which the bile ducts are destroyed by a progressive fibro-inflammatory process. BA is the most common cholestatic liver disease in children and is the leading indication for pediatric liver transplantation worldwide. The etiology of BA is unknown, but recent studies indicate that hepatic infiltration by oligoclonal populations of CD4(+) and CD8(+) T-cells plays a central role in pathogenesis of the disease. The IFN- γ inducible chemokine CXCL10, known for its involvement in recruiting lymphocytes to the liver, is highly up-regulated in BA. We hypothesized that CXCL10 promotes hepatic T-cell infiltration, and thus bile duct destruction, in BA. The only mammalian model of biliary atresia is Rhesus rotavirus (RRV) induced biliary injury in mice. In this study, we investigated the effects of Cxcl10 deficiency in the pathogenesis of biliary atresia in the RRV murine model. Newborn wild-type and Balb/c *Cxcl10*^{-/-} Balb/c pups were injected with 2.5×10^6 ffu of RRV intraperitoneally (IP) to induce biliary atresia. The pups were weighed at 48 hour intervals and evaluated for jaundice, pale stools, bilious urine, and survival. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TB) were measured from serum. Harvested livers were analyzed histologically to evaluate the extent of inflammation, necrosis, and architectural change. Balb/c *Cxcl10*^{-/-} mice demonstrated an improved phenotype and 50% greater weight gain (6.01 vs 3.99 gm at P12; $p=0.006$). *Cxcl10*^{-/-} mice also displayed decreased liver transaminases and less hyperbilirubinemia. ALT in *Cxcl10*^{-/-} mice was reduced by 55% (57.5 vs 126 U/L; $p=0.002$) at P8, although it indistinguishable from wild-type by P12. TB was also lower by 34% (7 vs 10.6 mg/dL; $p=0.02$) at P8 and decreased by 63% (6.7 vs 17.6 mg/dL; $p=0.02$) at P12. Histological evaluation revealed less biliary and portal tract inflammation compared to controls. These observations support the hypothesis that CXCL10 plays a vital role in the pathogenesis of biliary atresia through interaction with T lymphocytes. Strategies to target this chemokine or its receptor CXCR3 early in the development of biliary atresia may be therapeutic or circumvent the need for liver transplantation.

M Rhue

Title Therapeutic effect of increased hepatic lysosomal acid lipase in a murine model of non-alcoholic fatty liver disease (NAFLD)

INTRODUCTION: There are limited therapeutic options for NAFLD. Lysosomal acid lipase (LAL) is the critical enzyme for the hydrolysis of triglycerides and cholesteryl esters delivered to the lysosome. We hypothesized that increased hepatic LAL expression would improve the NAFLD lesion in our mouse model.

METHODS: Ldl receptor knock-out mice (*ldlr*^{-/-}) develop progressive hepatic steatosis and fibrosis when placed on a western diet. Using a doxycycline-inducible tet-off LAP-tTA mouse model system, the absence of doxycycline induces the expression of hepatic LAL; these mice were bred into a *ldlr*^{-/-} background (LAP-tTA/(tetO)₇-hLAL;*ldlr*^{-/-}). LAP-tTA/(tetO)₇-hLAL;*ldlr*^{-/-} (LAP) and *ldlr*^{-/-} male mice were analyzed after 2, 4, and 5 months of western diet; male wild type and *ldlr*^{-/-} mice also served as age-matched, standard diet controls. We collected body and liver weight data and performed hepatic LAL enzyme activity assays; hepatic sections were examined after H&E, Oil-Red-O, and Masson's trichrome staining.

RESULTS: The hepatic LAL activity level of the LAP mouse group is 2-2.8 times higher than the other mouse groups ($p < 0.0001$). After 4 months of western diet, the mean body weight of the *ldlr*^{-/-} and LAP mice are higher than that of the standard diet mice ($p < 0.0001$). Although there is no statistically significant difference between their body weights, the mean liver weight is lower in the western diet LAP compared to the western diet *ldlr*^{-/-} at 4 and 5 months ($p = 0.002$). After 4 months, the liver-to-body weight ratio is higher in the western diet *ldlr*^{-/-} mice than the western diet LAP mice ($p = 0.002$) and standard diet mice ($p = 0.029$). The western diet LAP mice have less hepatic steatosis and less hepatic fibrosis than the western diet *ldlr*^{-/-} mice at each time point. There is no significant difference between the standard diet *ldlr*^{-/-} and wild type mice.

CONCLUSION: Increased hepatic LAL expression delays the progression of hepatic steatosis and fibrosis in our model. LAL should be further explored as a potential enzyme therapy for NAFLD.

ABSTRACT

Meghana Sathe

ATP in bile is a potent secretagogue, stimulating biliary epithelial secretion through apical purinergic receptors. While biliary epithelial cells release ATP into bile, the mechanism is unknown. Indirect evidence in other cells suggests vesicular exocytosis contributes to epithelial ATP release. The aims of this study in live biliary epithelial cells were to i) determine if an ATP-enriched vesicular pool exists, ii) measure dynamic changes in this vesicle pool in response to known stimuli of ATP release, and iii) characterize the regulatory pathways involved.

METHODS: Studies were done in human Mz-Cha-1 cells and SV40-immortalized mouse large (MLC) and small cholangiocytes (MSC). ATP release was measured by luciferin-luciferase assay and reported as arbitrary light units (ALU). Quinacrine was used to localize intracellular ATP stores and FM4-64 to identify the endocytic compartment. Confocal microscopy with 3-D reconstruction and automated detection algorithm was used to measure dynamic changes in vesicle number.

RESULTS: ATP-enriched vesicles were present in all cells. ATP vesicles were distinct from the endosomal compartment, ranging in size from 0.4 to >1 μm . In response to cell swelling (33 % hypotonic exposure) relative ATP increased by 86 ± 15 ALU in Mz-Cha-1 cells (n = 7), 56 ± 25 ALU in MLC (n = 4), and by 19 ± 7 ALU in MSC (n = 6). ATP release was accompanied by decrease in ATP-enriched vesicles by 61% in Mz-Cha-1, by 57% in MLC, and by 65% in MSC versus only 5-14% in control cells exposed to isotonic buffer (n = 4-8, $p < 0.001$). Incubation of Mz-Cha-1 cells with Brefeldin, which disrupts secretory vesicle formation, significantly decreased the basal number of ATP-enriched vesicles by 21% (n = 8, $p < 0.001$) and prevented loss of vesicles (decrease of 4% vs 26% in control, n = 4-8, $p < 0.001$), while Nocodazole, which disrupts microtubules, increased the basal number of ATP-enriched vesicles by 39% (n = 8-10, $p < 0.001$), but prevented the loss of vesicles in response to hypotonic exposure (decrease of 6% vs 43% in control, n = 4-8, $p < 0.001$).

CONCLUSION: These imaging studies demonstrate for the first time the existence of a dynamic ATP-enriched vesicular compartment in biliary epithelial cells. ATP-enriched vesicles are distinct from endosomes and undergo microtubule-dependent regulated changes in response to cell swelling. Understanding the mechanisms involved in biliary epithelial cell ATP release may suggest strategies to increase ATP in bile, thereby augmenting biliary secretion and flow in the treatment of cholestatic liver disorders.

Title: Human embryonic stem cells as a research model for cell biology in health and disease.

Niemann Pick Type C (NPC) is a rare but lethal autosomal recessive disease caused by a mutation in NPC1, a housekeeping protein residing in the late endosomal compartment with a putative role in cholesterol transport. The result is a severe lipidoses with massive lysosomal accumulation of sterols and other lipids that ultimately cause cell death. The liver is a primary site of cholesterol accumulation in NPC and children often present with liver dysfunction. However, the most striking feature of the disease is neuronal death despite lack of obvious accumulation of cholesterol in the brain which suggests an alternate but yet unclear mechanism underlying cell death in NPC. Currently there is no cure or treatment for the disease and affected children die in their childhood or early adolescence. The disease is of enormous basic science interest as well as a hallmark model of dysfunctional cholesterol trafficking. Animal models are available but they fail to accurately mimic human pathology, and obtaining human tissue for research purposes is obviously difficult. A new and powerful tool to solve this issue is provided by human embryonic stem cells (hESC). These remarkable cells have the potential to differentiate into tissues from all three germ layers allowing the creation of genetically stable and proliferative models to study human disease.

We propose a model in which cholesterol accumulation in NPC is caused by impaired movement of the late endosome to sites of transfer or delivery of cholesterol. I postulate that NPC1 directly or indirectly mediates this movement through its effects on targeting and/or docking of vesicular bodies. In this model cell death would be caused by dysfunctional vesicular transport rather than by cholesterol accumulation.

To test my hypotheses I have used gene silencing to develop a set of independent and stable hESC lines that show different levels of NPC1 knockdown. These hESC lines have typical stem cell morphology, express pluripotency markers, are genetically normal and are capable to differentiate into cells of all three germ layers. NPC1 knockdown hESC survival is unaffected in the short term but these cells display signs of dysfunctional vesicular trafficking evidenced by accumulation of cholesterol and an abnormal pattern of lysosomal staining. We have therefore developed a novel human model of NPC that will allow us to understand the role of NPC-1 in health and disease and the dynamics of cholesterol trafficking in living cells.

Investigator's statement (check all that apply):

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This research involves the use of laboratory animals. IACUC # is _____.

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Safety and Efficacy of Adalimumab in Pediatric Patients with Crohn's Disease

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Abstract:

Objectives: Adalimumab has recently become available for adult Crohn's Disease (CD) patients as a viable alternate TNF- α inhibitor to Infliximab. To date, there have been no studies reviewing the use of Adalimumab in pediatric patients with CD. Our aim was to examine the safety and efficacy of Adalimumab therapy in pediatric CD patients.

Methods: We performed a retrospective chart review of 15 pediatric patients with CD who received Adalimumab at a single institution between January 2003 and March 2007. All patients had a prior history of an attenuated response or anaphylaxis to Infliximab. Each patient's chart was reviewed for age at diagnosis, gender, extent of disease, age at start of Adalimumab therapy, course of therapy, side effects noted during therapy, concurrent medications, and response to Adalimumab. Clinical response to Adalimumab was classified as complete, partial, or non-response based on the patients' ability to be weaned from steroids, increased or decreased need for steroids, or need for surgery during their course of treatment. This study was approved by the Cleveland Clinic Institutional Review Board.

Results: 15 pediatric patients with CD received Adalimumab over a 33 month period. 14 patients had adequate follow-up and one patient was lost to follow up. The mean age at initiation of therapy was 16.6 years ($r=10.3-21.8$ yrs., $SD\pm 3.1$ yrs.). The majority of patients received a 80 mg loading dose administered subcutaneously and 40 mg doses subsequently every 2 weeks. The average duration of therapy was 10.8 months ($r=1-25.5$, $SD\pm 8.3$). A total of 272 injections were given. Of the 14 patients with sufficient data for follow up, 7 (50%) had a complete response, 2 (14%) had a partial response, and 5 (36%) had no response to Adalimumab. Complete response was achieved after a mean of 5 injections ($r=3-11$, $SD\pm 2.8$). Five of the 14 patients with adequate follow up had fistulizing disease; three of these maintained fistula closure, one had temporary closure, and one patient required surgery to assist with closure. Twenty six adverse events occurred during therapy. Eight (57%) patients had at least one adverse effect. The most common events were abdominal pain and nausea. No serious adverse events were reported and no adverse events required discontinuation of Adalimumab.

Conclusion: Adalimumab was well tolerated in pediatric CD patients. Sixty-four percent of patients had a complete or partial response. No serious adverse events occurred during therapy. Additional studies are needed to evaluate the efficacy and determine optimal dosing of Adalimumab in the pediatric population with CD.

Human Alpha Defensin 5 mRNA Levels are Decreased in Children with Untreated, Newly Diagnosed Crohn Disease

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Introduction: Paneth cell defensins, including human alpha-defensins 5 (HD5) and 6 (HD6), are key effectors of the intestinal innate immune system. Prior studies have demonstrated decreased HD5 and HD6 levels in adult patients with established ileal Crohn's disease (CD), suggesting that decreased defensin levels may initiate and perpetuate inflammation in susceptible individuals. To circumvent the potentially confounding factors of longstanding disease duration and anti-inflammatory treatment effects in these studies, we measured HD5 and HD6 mRNA levels in the ileum of untreated children with newly diagnosed CD.

Methods: Patients ages 7 to 18 years undergoing first-time colonoscopy for any reason were approached for study participation. Mucosal pinch biopsies from the terminal ileum (TI) were obtained in addition to standard clinical TI and colonic biopsies. RT-PCR analysis of ileal tissue samples was performed to quantify Paneth cell HD5 and HD6 as well as sPLA2 and lysozyme, two antimicrobial peptides also produced by intestinal Paneth cells. Levels of the neutrophil chemoattractant IL-8 were assayed to assess inflammation. GAPDH, a constitutively expressed glycolytic enzyme, was used as an internal control. Each of the biomarkers was standardized to GAPDH levels. Ileal defensin levels from patients with normal ileal histology and no other pathologic diagnosis (controls) were compared to patients with CD ileitis using standard t-tests. Diagnosis of CD was made according to standard diagnostic criteria.

Results: Terminal ileal biopsies from 8 patients with CD (5 female) and 17 patients with no pathologic diagnosis (10 female) were analyzed. Average age of CD patients was 13.6 years (range 9-18 years). Average age of control patients was 14.8 years (range 8-18 years). In children with ileal CD, ileal HD5 mRNA levels were 65% of control values ($p=0.04$). Ileal HD6 mRNA levels in patients with CD ileitis were 89% of control values ($p=0.68$). sPLA2 and lysozyme levels were 1.6 and 2 times higher in CD ileitis than in the control group ($p=0.01$ and $p=0.004$ respectively). IL-8 levels were increased 42-fold in the CD ileitis group ($p=0.002$).

Conclusions: This is the first study to compare terminal ileal defensin levels in children with ileal CD at the time of diagnosis. HD5 mRNA levels are lower in children with newly diagnosed, untreated ileal Crohn's disease than in normal control patients, perhaps contributing to the immunopathogenesis of pediatric CD. Given an increase in sPLA2 and lysozyme mRNA levels in these CD patients, the decrease in HD5 is likely not explained by decreased Paneth cell mass.

Rate of Recurrence of Clostridium Difficile in Pediatric Patients with Inflammatory Bowel Disease.

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BACKGROUND: The incidence and associated morbidity of *Clostridium difficile* (CD) infection has been increasing at an alarming rate in North America. *Clostridium difficile* associated diarrhea (CDAD) is the leading cause of nosocomial diarrhea in the USA. Patients with CDAD have longer average hospital admissions and additional hospital costs. Evidence has demonstrated that patients with inflammatory bowel disease (IBD) have a higher incidence of CD in comparison to the general population. The aim of this study was to compare the rate of recurrence of CD in hospitalized pediatric patients with IBD compared to hospitalized controls. The secondary aim was to evaluate whether infection with CD resulted in a more severe disease course of IBD.

METHODS: This was a nested case control retrospective study of hospitalized pediatric patients. Diagnosis of CD was confirmed with stool Toxin A and B analysis. The following data was obtained from the medical records: demographic information, classification of IBD including location of disease, IBD therapy, and prior surgeries. In addition prior hospital admissions within 1 year and antibiotic exposure were recorded. The same information was recorded following CD infection.

RESULTS: A total of 138 patients with IBD and 80 control patients were included. The rate of recurrence of CD in the IBD population was 43% compared to 7.5% in the control population ($p < 0.0001$). In evaluating the effect on IBD disease severity, 57% of patients were readmitted with an exacerbation of disease within 6 months of infection with CD and 67% required escalation of therapy following CD infection. Of the patients with IBD, 44% of the cases were new onset IBD, 63% were on immunosuppression therapy and 33% were on gastric acid suppression prior to infection. In comparing the two populations, there was no significant difference in antibiotic exposure, 33% of IBD patients and 26% of control patients were on antibiotics, ($p < 0.2$). In regards to prior hospitalization, 10% of patients with IBD patients were hospitalized in the 30 days prior to infection in comparison to 27% of the control patients ($p < 0.002$).

CONCLUSION: CD infection in patients with IBD results in higher rate of recurrence and is associated with higher morbidity than the general population. Patients with IBD often required hospitalization and escalation of therapy following infection with CD, indicating that CD resulted in increased severity of disease. In addition, IBD patients were more likely develop community acquired CD, while the control patients developed noscomial infections, indicating a higher susceptibility to CD infection in patients with IBD.

Title: Clinical Outcome of Children with IBD and Preferential MMP Metabolism: Allopurinol vs. Alternative Therapy

Background: Azathioprine (AZA) and 6-mercaptopurine (6MP) are well established treatments for adults and children with inflammatory bowel disease (IBD). However, up to 40% of patients do not respond, at times because of an individual's preferential metabolism of the thiopurine to an inactive and potentially hepatotoxic metabolite, 6-methylmercaptopurine (6MMP), rather than the active 6-thioguanine nucleotides (6TGN) (Dubinsky et al., 2002). The use of allopurinol in AZA/6MP treated adults with preferential 6MMP production has improved levels of 6TGN, reduced 6MMP and been associated with clinical improvement (Sparrow et al., 2005, 2007). Parents of children with IBD and preferential 6MMP metabolism have been offered the opportunity to initiate similar therapy for their children when clinical circumstances warranted a change in therapy. We describe the clinical outcomes of children receiving allopurinol with low dose AZA/6MP versus alternative therapies.

Methods: Retrospective chart review identified children who were poor responders to AZA/6MP and had preferential production of 6MMP (defined as 6MMP/6TGN ratio >11) (Dubinsky et al., 2002). Poor AZA/6MP response was defined as any of the following: 1) persistent IBD-related symptoms, 2) elevated transaminases 3) steroid dependence. Subjects were grouped according to therapeutic intervention (Grp1: allopurinol and reduced dose AZA/6MP; Grp2: other therapies). Clinical goals for each subject were defined at initiation of therapy and response defined as achieving the goals by 3-6 months after beginning treatment. Thiopurine metabolites pre and post therapy were also determined.

Results: 27 children had 6MMP/6TGN >11 (mean age 12.4 yrs, 63% male, 20 Crohn, 7 UC). 14 received allopurinol (Grp1), and 13 (Grp2) alternative therapies (infliximab, methotrexate, surgery, adjusted 6MP/AZA dose, 5ASA). Clinical goals were achieved in 12/14 Grp1 subjects and only 4/13 Grp2 subjects (p<0.01). In Grp 1, one subject discontinued treatment due to leucopenia, and one required surgery due to persistently active disease. Four subjects in Grp 2 discontinued the initial change in therapy due to persistently active disease. All 4 steroid dependent Grp1 subjects successfully discontinued steroids by 6 months, compared to 0/2 in Grp2. Similarly, transaminases normalized in 6/7 Grp1 vs 4/8 Grp2. Lab findings pre and post treatment are summarized in the Table.

Conclusion: The combination of allopurinol and low dose AZA/6MP effectively achieves clinical goals in children with IBD and preferential 6MMP metabolism. Children on alternative therapy have a less favorable pattern of improvement.

Thiopurine Metabolite Levels Pre- and Post- Treatment

	6MMP (pmol/8 x 10 ⁸ RBC)		6TGN (pmol/8 x 10 ⁸ RBC)		6MMP/6TGN	
	PreRx	PostRx	PreRx	PostRx	PreRx	PostRx
Grp1	9811±4910	236±668*	179±54	308±93**	56±25	0.71±1.89***
Grp2	10,007±4853	8224±5859*	181±86	184±57**	61±30	43±25***

Grp1 vs. Grp2: *P=0.0022; **P=0.0146; ***P=0.0010

Investigator's statement (check all that apply):

This research involves human subjects research. The IRB approval # is 08-140.

This research involves the use of laboratory animals. IACUC # is _____.

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Plasma Citrulline Levels in Pediatric Patients with Small Bowel Crohn's Disease-A Novel Biomarker of Inflammation

Orhan Atay

Abstract

Objectives: The amino acid citrulline is primarily synthesized in small intestine enterocytes. Plasma citrulline is decreased in diseases with diminished mass of functional small bowel enterocytes, such as short bowel syndrome. The purpose of this study was to determine whether plasma citrulline concentrations are decreased in pediatric patients with active small bowel Crohn's disease (CD). There are no prior data regarding plasma citrulline levels in pediatric CD.

Methods: 28 patients with small bowel CD and 25 healthy control subjects were recruited from the Cleveland Clinic Children's Hospital. The Pediatric Crohn's Disease Activity Index (PCDAI) was determined for all patients and demographic and auxologic data were collected for all subjects. Plasma citrulline levels were determined using the Hitachi L-8800 Amino Acid Analyzer, using blood specimens obtained after an overnight fast. CD and control patients were compared on categorical and continuous factors using Chi-square, Fisher's exact, or Wilcoxon rank sum tests as appropriate. The association between plasma citrulline levels and continuous characteristics was assessed using Spearman correlation coefficients for all subjects and separately for each cohort.

Results: Patients with small bowel CD had a median age of 15.1 years (range: 9.2– 18.2 years); the median age of controls was 13.9 years (range: 9.2–18.6 yrs). Plasma citrulline levels did not differ significantly between males and females. However, CD patients had significantly lower plasma citrulline levels (median: 21 vs 33 micromolar; $p < 0.001$) and lower BMI (median percentile: 36 vs 73; $p = 0.037$) compared to age-matched controls. There also was an inverse relationship between PCDAI scores and plasma citrulline levels. In addition, there was correlation between the PCDAI score and BMI in CD patients, implying that a lower BMI indicates more severe disease status.

Conclusion: Pediatric patients with small bowel CD have significantly lower plasma citrulline levels than age-matched controls. The PCDAI scores in CD patients inversely correlate with their plasma citrulline concentrations. These data suggest that determination of plasma citrulline may be a non-invasive marker of small bowel inflammation in pediatric patients with small bowel CD.

TITLE: Evaluation of Intestinal Fibrosis in the TNBS Rat Model of Crohn's Disease Using Ultrasound Elasticity Imaging (UEI).

AUTHORS: *Rangwalla, S.C.; Johnson, L.A.; Rubin, J. M.; Kim, K.; Congxian, J.C., Higgins, P.D.R.

BACKGROUND: Repeated cycles of inflammation and healing in Crohn's disease lead to fibrosis. No reliable non-invasive methods of detecting fibrosis currently exist. Repeated TNBS (2, 4, 6-trinitrobenzene sulfonate) enemas in the rat colon result in recurrent inflammation and fibrosis similar to Crohn's disease in humans. UEI uses ultrasound imaging along with speckle tracking to measure the stiffness of tissues.

AIMS: 1) To use *in vivo* UEI to compare fibrotic regions of the colon to non-fibrotic regions of the colon.
2) To validate *in vivo* UEI by testing the correlation of *in vivo* UEI measurements of strain with *ex vivo* mechanical measurements.

METHODS: Female Lewis Rats (150g-180g) were used for this experiment. Colonic TNBS enemas were administered to 6 rats and as a control, phosphate buffered saline (PBS) enemas were administered to 5 rats. Enemas were given once weekly over a 5 week period, and a one week rest period before sacrifice was used to minimize residual edema. A commercial ultrasound scanner (Phillips iU22) with a 12 MHz linear array transducer was used to obtain images. Images were obtained from the ascending colon and from the sigmoid colon. Colonic segments were deformed by compressing them between the transducer applied to the anterior abdominal wall and the incompressible spine. Ultrasound radiofrequency (RF) data was collected and processed using a 2-D correlation-based phase-sensitive speckle tracking algorithm to derive a measure of tissue strain. To account for differences in compression between pushes, the strain values were normalized to the average strain measured between the transducer and the spine. Tissue from the ascending (non-fibrosed) and sigmoid colon (fibrosed in TNBS only) segments had Young's modulus measured *ex vivo* with a MicroElastometer (Artann Labs). Between-treatment comparisons were made using the difference between the proximal and distal colon strain (measured by *in vivo* UEI) in each rat. Linearity of the *ex vivo* stress-strain measures was also evaluated to determine the cause of the tissue stiffness.

RESULTS: Distal to proximal differences in normalized strain were compared using two-tailed t-test. The mean change in normalized strain was 2.37 (SD 0.58) in TNBS rats and -0.24 (SD 0.58) in PBS rats ($p = 0.00005$). *Ex vivo* mechanical measurement of Young's modulus correlated with UEI strain measurements ($\rho = 0.6755$; $p = 0.0006$). Stress-strain curves were highly nonlinear, suggesting that tissue stiffness was due to mechanical properties of the tissue rather than edema.

CONCLUSION: Ultrasound elasticity imaging (UEI) can detect fibrosis in a rat TNBS model of chronic intestinal fibrosis. UEI strain measurements differentiated fibrotic from non-fibrotic segments of intestine. UEI strain measurements also correlated well with the *ex vivo* gold standard of for tissue stiffness, Young's modulus. This suggests that UEI could be used as a tool to detect fibrosis in humans with Crohn's disease.

GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR IS REQUIRED FOR HOMEOSTATIC RESPONSES TO INTESTINAL INJURY IN THE *CARD15* DEFICIENT HOST.

Samson, Charles M.; Jurickova, Ingrid; Colliver, Joshua; Bonkowski, Erin L.; Han, Xiaonan; Denson, Lee.

Background: We have identified a subset of Crohn Disease (CD) patients with neutralizing antibodies to Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF Ab) who exhibit defective neutrophil function and increased risk for stricturing ileal behavior. While polymorphisms in *CARD15/NOD2* are associated with CD, the population attributable risk is low, suggesting the requirement for additional risk factors. We **hypothesized** that gm-csf neutralization would impair homeostatic responses to gut injury in *card15* deficient mice.

Methods: 4 week old C57Bl/6J wild type (WT) or *card15* deficient (C15KO) mice were injected with IgG or neutralizing gm-csf Ab. Two weeks later, mice were placed on piroxicam (NSAID, 200 ppm) chow for 1 week, and ileal histology was assessed. Mesenteric lymph nodes (MLN) were harvested for FACS analysis of dendritic cells (DC, CD11c^{hi}), macrophages (mΦ, F4/80⁺CD11b⁺), effector T cells (Teff, CD4⁺CD44⁺CD62L⁻), and regulatory T cells (Treg, CD25⁺Foxp3⁺). Intracellular staining was used to detect cytokines in DC and mΦ (IL-6/IL-10) and T cells (IL-4/IL-17/IFN γ). Statistical analysis was performed with t-tests (n>5/group and p<0.05 significant). **Results:** Under basal conditions, both IL-6 (9%±2 vs.20%±4, p<0.04) and IL-10 (6%±4 vs.20%±5, p<0.03) producing DCs were reduced in C15KO mice compared to WT. A similar reduction in basal mΦ production of IL-6 and IL-10 was observed in C15KO mice. NSAID exposure induced mild ileal injury in both WT and C15KO. Under these conditions, C15KO, but not WT, demonstrated an expansion of IL-6 and IL-10 producing DCs, and IL-4 (16%±4 vs.7%±1), IL-17 (5.9%±0.7 vs.3.5%±0.8, p<0.04), and IFN γ (3.4%±0.4 vs.1.7%±0.6, p<0.02) producing Teff. The frequency of Treg increased significantly in both WT (6.9%±0.6 vs.23%±2, p<0.04) and C15KO (12.2%±0.6 vs.22%±1, p<0.0001). Following gm-csf neutralization, ileal injury was substantially increased in C15KO compared to WT. Under these conditions Teff increased in both WT (19±1% vs.11±2%, p<0.01), and C15KO (25±16% vs.10±4%, p<0.02), and Treg expansion was abrogated. The increase in IL-10 producing DC in C15KO mice with NSAID exposure was completely prevented by gm-csf neutralization (21%±3 vs.11%±2, p<0.03), and did not change in WT controls. **Conclusions:** Transmural ileitis in *card15* deficient mice following gm-csf neutralization and NSAID exposure is associated with a reduction in IL-10 producing DCs and Tregs and a mixed expansion of Th1/Th2/Th17 cells. Our data suggest that gm-csf is required for homeostatic responses to gut injury in the *card15* deficient host, and that therapeutic GM-CSF administration may be of particular benefit in this setting.

Investigator's statement (check all that apply):

This research involves human subjects research. The IRB approval # is _____.

This research involves the use of laboratory animals. IACUC # is 6E0754_____.

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STAT6 Activation in Ulcerative Colitis: A Target for Prevention of IL-13-Induced Colonic Epithelial Cell Dysfunction

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Background & Aims: IL-13 has been implicated as an effector cytokine in the pathophysiology of ulcerative colitis (UC). Binding of IL-13 to its receptor leads to activation of STAT6. However, the STAT6 phosphorylation status in patients with UC is unknown, as is the effect of STAT6 inhibition on colonic epithelium exposed to IL-13. Therefore, the aim of this study was to determine if STAT6 phosphorylation is increased in patients with UC, and if inhibition of STAT6 activation attenuates IL-13-induced apoptosis and expression of the pore-forming tight junction protein claudin-2 in human colonic epithelial cells. **Methods:** To assess IL-13 signaling, immunohistochemical staining for phosphorylated (p) STAT6 was performed on paraffin embedded colonic tissue from pediatric subjects with UC (early UC, n=10) and Crohn's disease (CD, n=10) at diagnosis, colectomy tissue from adults with UC (late UC, n=5) and controls (n=10). A pathologist blinded to the diagnosis scored histological sections on a 0-4 scale for pSTAT6. In cell culture experiments, HT-29 cells were pre-treated with vorinostat (1 or 5 μ M), an FDA-approved histone deacetylase inhibitor that has been shown to inhibit STAT6 activation in a lymphoma cell line, or vehicle control prior to treatment with IL-13 (10 ng/ml). Western blot analysis was performed on cellular lysates for total and pSTAT6, and for claudin-2. Apoptosis was determined by flow cytometry of Annexin V-PE-stained cells and confirmed with immunodetection of cleaved caspase-3. **Results:** Median score for epithelial pSTAT6 was 0 in control subjects, 2 in early UC (vs. control $P=0.004$), 4 in late UC ($P=0.014$), and 0 in CD. In HT-29 cell culture, vorinostat inhibited IL-13-induced STAT6 phosphorylation. IL-13 increased claudin-2 expression 3.5-fold, which was inhibited by vorinostat. Treatment with IL-13 for 48 hours increased apoptosis 2.7-fold ($3.7\pm 1.6\%$ vs. $10.0\pm 1.4\%$ Annexin V+, $P=0.007$). Vorinostat treatment alone did not increase apoptosis but reduced IL-13-induced apoptosis in a dose-dependent manner with complete suppression to baseline at 5 μ M (IL-13 + vorinostat 1 μ M $7.5\pm 0.8\%$, + 5 μ M $4.8\pm 0.9\%$, $P_{trend} < 0.001$). Immunodetection of cleaved caspase-3 demonstrated the same effect. **Conclusions:** Increased STAT6 phosphorylation in the colonic epithelium of pediatric subjects with presenting colonoscopies for UC supports an effector role of IL-13 early in the disease. Treatment of colonic epithelial cells with vorinostat, a drug that inhibits IL-13-induced STAT6 activation, reduces IL-13-induced apoptosis and claudin-2 expression suggesting that STAT6 may be a novel therapeutic target for UC.

Investigator's statement (check all that apply):

[X] This research involves human subjects research. The IRB approval # is 070747.

[] This research involves the use of laboratory animals. IACUC # is _____.

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THE ROLE OF *ATG16L1* IN BACTERIAL-INDUCED AUTOPHAGY

S Hussey, L Yuan, L Travassos, DJ Philpott and NL Jones

Background: Recent genome wide association studies have implicated variants of the autophagy gene *ATG16L1* in Crohn disease pathogenesis. However, functional studies translating this risk association are lacking to date.

Aims: The aim of this study was to validate the role of *ATG16L1* in autophagy induction *in vitro* in response to the intracellular pathogen *Shigella flexneri* and bacterial associated ligands.

Methods: Epithelial cells (MDAMC) stably transformed to produce green fluorescent light chain 3 (GFP-LC3), a known marker of autophagy, were transduced with lentiviral short hairpin RNA (shRNA) constructs, interfering with *ATG16L1* expression. Following transduction, cells were examined for their ability to control infection of wild-type (WT) or IcsB mutant (Δ IcsB) *S. flexneri* using gentamicin protection assays. WT *S. flexneri* is reported to evade autophagy whereas Δ IcsB *S. flexneri* is more susceptible to autophagy. Cells treated with rapamycin ($15\mu\text{g}/1\times 10^6$ cells) served as positive controls for autophagy. Lymphoblast cell lines (LCLs) from participants in the HapMap project who were homozygous for either risk (G) or protective (A) *ATG16L1* alleles were transduced with a GFP-LC3 expressing lentivirus construct and subsequently treated with rapamycin, muramyl dipeptide (MDP) and peptidoglycan (PG). Autophagy induction was again assessed using confocal microscopy.

Results: Suppression of *ATG16L1* using shRNA constructs was confirmed by immunofluorescence microscopy and *ATG16L1* antibody by Western blotting. *ATG16L1* suppression resulted in a profound reduction of autophagy induction following rapamycin treatment. Autophagosome formation around either WT or Δ IcsB *S. flexneri* was almost completely suppressed in shRNA transduced cells compared with control cells. Autophagosome formation in LCLs was also observed following both MDP and PG treatments. However, cells from a GG lineage had a mean 34% less autophagosomes/cell compared with AA cells, indicating a less efficient autophagy response to these bacterial ligands.

Conclusion: This study demonstrates that *ATG16L1* is essential to autophagosome formation in response to intracellular bacterial infection in epithelial cells. We also reveal a reduced autophagy response to NOD receptor ligands in myeloid cells homozygous for the Crohn disease associated (G) *ATG16L1* allele, which may be of importance in disease pathogenesis.

Investigator's statement (check all that apply):

- This research involves human subjects research. The IRB approval # is _____.
- This research involves the use of laboratory animals. IACUC # is _____.

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Foxp3-dependent beneficial effects of DNA methyltransferase inhibitor therapy on murine colitis

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Background & Aims: Foxp3⁺ regulatory T cells (Tregs) are potent modulators of cellular immune responses, and epigenetic modification of Foxp3 gene expression and function using histone deacetylase inhibitors ameliorates murine inflammatory bowel disease (IBD). Given that a highly conserved region in intron 1 of the human and murine Foxp3 locus is subject to methylation, we tested the effects of DNA methyltransferase inhibitor (DNMTi) therapy in the dextran sulphate sodium (DSS) model of murine colitis to assess whether DNMTi use might promote Foxp3 gene demethylation, affect Treg activity and thereby modulate disease. ***Methods:*** DSS colitis was induced in unmodified C57BL/6 mice or in a subgroup in which Tregs were depleted by thymectomy plus CD25 monoclonal antibody therapy. Mice were injected daily with PBS or 0.1 mg/kg of a clinically approved DNMTi (5-aza-2'-deoxycytidine) from the onset of DSS administration or once colitis was induced. ***Results:*** The severe colitis induced using

DSS, involving marked weight loss, blood in the stool, diarrhea, colonic shortening and characteristic histologic changes, was significantly reduced by DNMTi therapy. DNMTi administration decreased expression of multiple inflammatory cytokines and T cell activation markers in secondary lymphoid tissues and in the lamina propria. However, DNMTi therapy was largely ineffectual when DSS colitis was induced in mice in which Tregs were depleted. ***Conclusions:*** We conclude that DNMTi therapy can modulate gene expression and function, and thereby offers a new therapeutic approach for therapy in IBD. The efficacy of DNMTi is at least in part dependent upon the actions of Foxp3+ Treg cells. Further studies are needed to further analyze the beneficial effects of DNMTi on Tregs and T effector cells in additional experimental models of colitis.

Immune response to influenza vaccine in children with inflammatory bowel disease

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OBJECTIVE: Patients with inflammatory bowel disease (IBD) frequently receive immunosuppressive therapy. The immune response in these patients to vaccines has not been well studied. We conducted a prospective, open label study to evaluate the serologic response to influenza vaccine in children with IBD.

METHODS: Serum was obtained from 146 children and young adults with IBD (96 CD, 47 UC, 3 IC) for baseline influenza titer, immediately followed by immunization with trivalent [A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 (B)] inactivated influenza vaccine. Subjects returned for repeat titers 3-9 weeks later. Seroprotection against each influenza strain was defined as hemagglutination inhibition (HAI) titer ≥ 40 . Patients were categorized as non-immunosuppressed [(NIS), aminosalicylates only, antibiotics only, or no therapy] or immunosuppressed [(IS), any immunosuppressive agent]. IS patients were further subcategorized as: (1) tacrolimus; (2) TNF-alpha inhibitor; (3) immunomodulator; and (4) corticosteroids only.

RESULTS: More patients were seroprotected against strains A/H1N1 and A/H3N2 than B strain ($p < 0.02$), regardless of immunosuppression status. The proportion seroprotected and geometric mean titers at post-vaccination were similar between NIS and IS groups for all three strains. Subanalysis of patients not seroprotected at baseline showed that those receiving anti-TNF therapy were less likely seroprotected against strain B (14%) compared to patients in the NIS group (39%, $p = 0.025$). There were no serious vaccine-associated adverse events.

CONCLUSION: Influenza vaccination produces a high prevalence of seroprotection in IBD patients, particularly against A strains. The vaccine is well tolerated. Routine influenza vaccination in IBD patients is recommended, irrespective of whether patients receive immunosuppressive medications.

Investigator's statement (check all that apply):

This research involves human subjects research. The IRB approval # is 07-09-0345.

This research involves the use of laboratory animals. IACUC # is _____.

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Surgery in a Prospectively Followed Cohort of Pediatric Patients with Crohn's Disease (CD)

Marc E Schaefer

Previous studies of the incidence of surgery in pediatric Crohn's Disease (CD) have been either retrospective, from a single (referral) center, or both. **AIMS:** To determine the incidence of intestinal surgery and CD-related surgery in an inception cohort of pediatric CD patients who have been diagnosed between 2002 and 2008 and to identify risk factors for surgery in CD.

METHODS: Patients \leq 16 years of age with newly diagnosed CD were enrolled in the Pediatric IBD Collaborative Research Group (PIBDCRG) Registry, a prospective, multi-center (26 site) observational study. Patients were managed according to the practice of their pediatric gastroenterologist. Uniform data were collected at diagnosis, 30 days, and then quarterly from 855 consecutively enrolled pediatric CD patients. **RESULTS:** Follow-up data were complete for 620 patients for 1 year, 430 patients for 2 years, 310 patients for 3 years, 184 patients for 4 years, 70 patients for 5 years, and 7 patients for 6 years. In all, 77 of the 855 patients underwent a first surgery, 57 intestinal surgeries (intestinal resection, ostomy, strictureplasty, or appendectomy) and 20 other surgeries (abscess drainage and fistulotomy). The estimated cumulative risk of intestinal surgery was 4% at 1 year after diagnosis, 7% at 2 years, 10% at 3 years, 12% at 4 years, and 14% at 5 years. The estimated cumulative risk of all CD-related surgery was 5% at 1 year after diagnosis, 8% at 2 years, 12% at 3 years, 16% at 4 years, and 18% at 5 years after diagnosis. The incidence of intestinal surgery and CD-related surgery in the first six months after diagnosis were 2.3% and 3.3% respectively and were each higher than any other subsequent six-month period. Older age at diagnosis, increase in disease severity, disease limited to the ileum and/or right colon, inflammatory disease changing to stricturing disease, prednisone exposure within three months, and an increase in the sum of positive serologic antibodies were associated with an increased risk of surgery. **CONCLUSIONS:** The estimated cumulative risk of requiring intestinal surgery for this inception cohort is less than the reported incidence in previous studies. The first six months after diagnosis has the highest probability of intestinal surgery or CD-related surgery than any other six-month period in the first five years after diagnosis. Disease severity, location, and behavior, as well as age at diagnosis, medication exposure, and immune reactivity are helpful in predicting the risk for surgery in pediatric CD. Whether changes in general practice patterns or in the use of specific interventions (i.e. greater use of immune-suppressants and/or biologicals) will affect future outcomes remains to be seen

Investigator's statement (check all that apply):

This research involves human subjects research. The IRB approval # is 0184-04.

This research involves the use of laboratory animals. IACUC # is _____.

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Title: The Role of Lectin-Like Transcript 1 (LLT1) in Celiac Disease

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Introduction/Background: Rosen et al and Aldemir et al described LLT1 as a ligand for the NKRP1A receptor in 2005. Since NKRP1A+T cells constitute 70% of T cells of jejunal intra-epithelial lymphocytes (IEL), we hypothesized that LLT1 may play a role in the pathogenesis of celiac disease.

Methods: Peripheral blood and duodenal biopsies were taken from active celiac patients, celiac patients on a gluten free diet (GFD), and control (non-celiac) patients undergoing upper endoscopy. Informed consent was obtained on all patients. Lymphocytes were isolated from both blood and intestinal tissue, and analyzed for LLT1 by real time PCR, immunohistochemistry (IHC), and flow cytometry.

Results: Real time PCR showed a 25-fold increase in LLT1 mRNA in IEL of one active celiac patient compared to one control. Patients on a GFD showed a decrease in LLT1 mRNA proportional to improvement in histology. This difference was not observed in peripheral blood lymphocytes. IHC demonstrated strong LLT1 staining of infiltrating lymphocytes on active celiac patients while minimal staining of enterocytes and lamina propria in control patients. Patients on a GFD had decreased LLT1 staining but not to the degree of control patients.

Conclusions: LLT1 expression is upregulated in patients with celiac disease suggesting that LLT1 may play an important role in local immunity in celiac disease.

**INFLIXIMAB-INDUCED PSORIASIS IN PEDIATRIC CROHN DISEASE;
EXPERIENCE OF THIS PARADOXICAL EVENT AT A TERTIARY CENTRE**

Mary Sherlock, Mary Zachos, Anne Griffiths.

Background: Infliximab is now an established therapy for pediatric Crohn disease (CD). It also has a role in the management of psoriasis, an autoimmune skin condition in which TNF alpha has a role in the pathogenesis. Since the introduction of anti-TNF therapy, there have been numerous cases reports documenting new-onset psoriasis and psoriasiform lesions following the use of these agents. The pathogenesis of this paradoxical process has not been fully elucidated.

Aims: This case series aims to highlight this unexpected side-effect of anti-TNF therapy. New onset psoriasis and psoriasiform lesions following initiation of anti-TNF therapy have been described in adult patients. However, to our knowledge, there has been only one published case report of psoriasis developing de novo in a child with CD following infliximab therapy. This study represents the largest case series describing this paradoxical skin condition in pediatric CD patients receiving infliximab therapy.

Methods: The SickKids IBD database was searched to identify all CD children who have ever received infliximab. The medical records of all patients with documented skin abnormalities during infliximab therapy were reviewed. Data regarding age at diagnosis, disease duration, family and personal history of psoriasis, and duration of infliximab exposure, was retrieved.

Results: 118 children received infliximab since 2000. Eleven children (9.3%), 7 male, developed new-onset psoriasis or psoriasiform lesions while receiving infliximab therapy. Persistent luminal disease despite conventional therapy (n=8) and perianal disease (n=3) were the indications for initiation of infliximab. The median age at diagnosis of psoriasis or psoriasiform lesions was 14.6 years (IQR 13.1 – 14.9). The median duration of infliximab exposure at the time of psoriasis onset was 1.03 years (IQR 0.57 – 2.07). The median dose of infliximab at the time of psoriasis onset was 5mg/kg (IQR 5 – 7.5) with a median time interval between infusions of 8 weeks (IQR 7 – 8). Six patients were receiving concomitant immunomodulation at the time of onset of the skin lesions. Features were consistent with plaque psoriasis in 9 patients and guttate psoriasis in 2 patients. No patient had a prior history of psoriasis and 2 patients had a positive family history. Ten of 11 responded well to topical steroid therapy. Two patients discontinued infliximab, one of whom switched to adalimumab without a return of the psoriatic skin lesions.

Conclusion: This case series highlights the importance of careful skin examination in all patients receiving infliximab therapy. This allows for early specialist referral and rapid treatment. Most patients respond well to topical therapy and in the majority of cases, anti-TNF therapy can be continued.

Investigator's statement (check all that apply):

- This research involves human subjects research. The IRB approval # is _____.
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Abstract

Background: Extraintestinal manifestations (EIMs) in pediatric patients with inflammatory bowel disease (IBD) are poorly characterized. We examined the prevalence of EIMs at diagnosis, subsequent incidence, and risk factors for EIMs.

Methods: Data for 1649 patients from the PedilBD Consortium Registry, diagnosed with IBD before 18 years of age [1007(61%) with Crohn's disease, 471(29%) with ulcerative colitis, and 171(10%) with indeterminate colitis], were analyzed using logistic regression, Kaplan-Meier, log rank tests and Cox models.

Results: EIMs were reported prior to IBD diagnosis in 97 of 1649 patients (6%). Older children at diagnosis had higher rates compared with younger children, and arthritis (26%) and aphthous stomatitis (21%) were most common. Among the 1552 patients without EIM at diagnosis, 290 developed at least one EIM. Kaplan-Meier estimates of cumulative incidence were 9% at 1 year, 19% at 5 years, and 29% at 15 years after diagnosis. Incidence did not differ by IBD type ($p=0.20$), age at diagnosis ($p=0.22$), or race/ethnicity ($p=0.24$). Arthritis (17%) and osteopenia/osteoporosis (15%) were the most common EIMs after IBD diagnosis.

Conclusions: In our large cohort of pediatric IBD patients, 6% had at least one EIM before diagnosis of IBD. At least one EIM will develop in 29% within 15 years of diagnosis. Incidence of EIMs both before and after diagnosis of IBD differs by type of EIM and may be slightly higher in girls, but is independent of the type of IBD, age at diagnosis, and race/ethnicity. age at diagnosis, type of inflammatory bowel disease or race. Clarifying patterns of extraintestinal anifestations in children with inflammatory bowel disease will help pediatric practitioners in identification and optimization of treatment for these patients

Investigator's statement (check all that apply):

- This research involves human subjects research. The IRB approval # is _____.
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