in fat. The gastrointestinal peptide cholecystokinin (CCK) is released from the duodenum in response to dietary fat. CCK has also been shown to stimulate growth of pancreatic cancer through the CCK receptor that is over-expressed on pancreatic cancer cells. The aim of this investigation was to determine if dietary fat promotes growth of pancreatic cancer through the actions of CCK at its receptor. METHODS/STUDY POPULATION: The effects of dietary fat on growth of murine Panc02 pancreatic cancer xenografts were studied in 3 different systems with immune competent mice: (1) pharmacologic blockade with a CCK receptor antagonist, (2) genetic knockout of the CCK receptor by CRISPR, and (3) in genetically engineered mice lacking the CCK peptide (CCK-KO). After injection of 2 × 106 Panc02 cells subcutaneously, mice were fed either a high-fat diet or a control diet for 37–42 days. Tumor volumes and weights were measured and histology performed. RESULTS/ANTICIPATED RESULTS: Dietary fat significantly increased the size of pancreatic cancer xenografts and this effect was reversed by CCK receptor blockade. Receptor antagonist therapy also significantly reduced tumor-associated fibrosis and increased the influx of CD8+ lymphocytes in the micro-environment. Panc02 cancer cells lacking CCK receptors failed to respond exogenous administration of CCK in vitro and to dietary fat in vivo. Dietary fat did not stimulate Panc02 tumor growth in CCK-KO mice. DISCUSSION/SIGNIFICANCE OF IMPACT: The mechanism by which dietary fat stimulates growth of pancreatic cancer is by CCK and this effect is independent of obesity. This is a significant finding because of the potential beneficial effects of medications which can block the effects of CCK in populations at risk for pancreatic cancer consuming a high-fat diet.

2298

Allergic asthma is associated with elevated sphingolipid levels in children

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OBJECTIVES/SPECIFIC AIMS: To determine if altered sphingolipid metabolism and composition are associated with childhood-onset asthma. METHODS/STUDY POPULATION: Sphingolipid profiles and composition were analyzed in a pilot cohort of pediatric with asthma (n = 22), and in nonasthmatic controls (n = 17). The cohort includes males and females, ages 5–17 years with no prior history of asthma or wheezing, and those who have been previously diagnosed with asthma by a pediatric pulmonologist. Subjects who have a history of prematurity, chronic lung disease, acute respiratory infection, malignancy, autoimmune disorders, immunodeficiency, or sickle cell anemia were excluded. Asthma and nonasthma phenotypes were determined through clinical history, standardized asthma symptom checklists, medical record review and spirometry. Masses of sphingolipids were quantified by mass spectrometry (HPLC-MS/MS) in serum and exhaled breath condensates (EBC). Allergy status was determined through clinical questionnaire, blood IgE (>150 IU/mL) and blood eosinophils (>0.3 × 103/mcl). RESULTS/ANTICIPATED RESULTS: Multiple species of sphingolipids and ceramides were found to be higher in the serum and EBC of asthmatics compared with controls in the overall cohort. In serum, these species include C16 ($p = 0.05$), C16DH ($p = 0.05$), C18:1DH ($p = 0.002$), C20 ($p = 0.05$), Sphingosine ($p = 0.05$), and S1P ($p = 0.04$). In EBC, asthma was associated with higher levels of C18:1DH ($p = 0.05$), C20 ($p = 0.05$), C22 ($p = 0.05$), Sphinganine ($p = 0.05$), Sphingosine ($p = 0.04$), and S1P ($p = 0.06$). When data were stratified for allergic status, the increases in serum sphingolipids were largely associated with total IgE levels greater than 150 IU/mL. Sphingolipids which were increased in allergic asthma (n = 13) compared with allergic controls (n = 5) included C16 ($p = 0.006$), C16DH ($p = 0.006$), C18:1DH ($p = 0.06$), C20 ($p = 0.048$), C22 ($p = 0.02$), C24 ($p = 0.02$), C24:1 ($p = 0.02$), Sphinganine ($p = 0.02$), Sphingosine ($p = 0.01$), and S1P ($p = 0.02$). Notably, only C18:1DH remained increased in asthmatics regardless of allergic status, in both low and high total IgE subjects. DISCUSSION/SIGNIFICANCE OF IMPACT: Data from this pilot cohort suggest that sphingolipids are altered in asthmatic compared with nonasthmatic children, particularly in association with a history of allergy and elevated blood IgE. This trend was also demonstrated in exhaled breath condensate, suggesting that sphingolipids are altered both in serum and airway fluid. Only 1 species of sphingolipid measured, C18:1DH, was elevated in asthmatics regardless of allergic status. Notably, this sphingolipid was recently identified to be associated with exercise induced wheezing (EIW) and asthma persistence overtime, in a large case-control study of children with and without asthma (Perzanowski *et al.*, in press). EIW has been identified as a specific phenotype of asthma, and can be present with or without allergy/atopy. Taken together, these data suggest that altered sphingolipids may contribute towards the underlying pathophysiology of asthma, the understanding of which can lead to improved characterization of asthma phenotypes.

Reference

Perzanowski M, *et al*. Distinct serum sphingolipid profiles among school-age children with exercise-induced wheeze and asthma persistence. *American Journal of Respiratory and Critical Care Medicine* 2017 (in press).

2299

Targeted eccentric motor control to improve locomotion after incomplete spinal cord injury

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OBJECTIVES/SPECIFIC AIMS: Incomplete spinal cord injury (iSCI) is a life-long disability that typically results in a profound loss of locomotion capability. Current rehabilitation methods rarely restore full community ambulation, which in turn limits quality of life. Most individuals with iSCI exhibit persistent deficits in eccentric muscle control and reach recovery plateaus below the levels necessary for independent community ambulation. Eccentric motor control is essential during the weight acceptance phase of gait, which is emphasized during downhill walking. METHODS/STUDY POPULATION: The overground locomotion of subjects with chronic iSCI was analyzed both prior to and following a 12-week downhill body-weight-supported treadmill training regimen and compared to that of matched healthy controls in terms of kinematics, kinetics, and EMG activation. RESULTS/ANTICIPATED RESULTS: We expect to find significant differences between the controls and subjects with iSCI, with deficits in eccentric motor control accounting for some of these differences. In addition, we expect the downhill training to yield significant improvement in eccentric muscle control that translates into improvements in functional, overground walking for the subjects with iSCI. DISCUSSION/SIGNIFICANCE OF IMPACT: The goal is to determine if downhill training can improve eccentric motor control and extend recovery beyond established plateaus. OpenSim modeling of the experimental data will help quantify changes in eccentric control of individual muscles to clarify where specific gains are made.

2325

Steroid therapy limits stem cell activation required to enact mucosal healing in inflammatory bowel disease

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OBJECTIVES/SPECIFIC AIMS: Intestinal stem cells (ISC) primarily act in the repair of ulcerated epithelium, and their proliferative capacity relies on Wnt/β-catenin signaling. However, the role of GCs on basal epithelial cell signaling has not been fully characterized. The objective of this study was to interrogate a mechanism by which steroids may limit ISC activation. GCs inhibit NFκB signaling, which has been shown to play a role in nuclear β-catenin activation in epithelial cells. We hypothesized that GCs limit Wnt/β-catenin signaling required for ISC activation and epithelial restitution by inhibiting NFκB activation in epithelial cells. METHODS/STUDY POPULATION: To examine the effects of GCs on intestinal epithelial cells, we treated a nontransformed human colonic epithelial cell line (NCM460) with dexamethasone and observed the effects on NFκB and Wnt/β-catenin signaling events. We isolated mouse epithelial cells from the distal colon for stem cell culture as 3D "organoids." We obtained pure epithelial cell preparations from mucosal biopsies isolated from patients treated at GI clinics at the University of Kentucky Chandler Hospital and VA Medical Center, Lexington. Steroid treated patients with equivalent levels of inflammation, but no mucosal ulceration were used as controls. RESULTS/ANTICIPATED RESULTS: In steroid-treated NCM460 cells, we saw an increase in steroid-responsive genes GILZ and SGK1. We saw a significant decrease in transcripts for Wnt target genes, including Axin2 and cmyc; NFκB target genes, including IFNG and IL6; and the shared NFκB and Wnt pathway co-activator CREBBP, despite unchanged transcript levels for β-catenin (CTNNB1). This data was corroborated in 3D stem cell cultures from cells isolated from mouse colon tissue, which had significant decreases in transcripts for stem cell markers Lgr5 and Ascl2, proliferative markers KI67 and PCNA, and Wnt target Axin2. NCM460s transfected with a lentivirus carrying a TCF/LEF luciferase construct showed a 2.5-fold decrease in TNF-stimulated luciferase activity with dexamethasone treatment. Interestingly, this effect can be rescued by glucocorticoid receptor (GR) blockade with RU-486. Intestinal epithelial cells from patient biopsies showed significant decreases in colitis-induced Axin2, p-LRP6 (a positive marker of Wnt Signaling) and nuclear β-catenin, which correlated with decreased p-p65 protein levels. DISCUSSION/SIGNIFICANCE OF IMPACT: Together, these data suggest that steroid therapy inhibits Wnt/β-catenin signaling at multiple levels, and effects stem cell proliferation in pure stem cell cultures. Decreases in TCF/LEF transcriptional activation (nuclear β-catenin's DNA binding target) can be reversed with steroid receptor blockade with RU-486, suggesting that a receptor level interaction may be occurring. Interestingly, the required co-activator CBP, shared between NFκB and Wnt pathways, has decreased transcription following steroid treatment, which may provide a mechanism for limited Wnt

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