whether supplementation with exogenous itaconate could ameliorate colitis. WT mice were treated with the cell-permeable form of itaconate, dimethyl itaconate (DMI). Administration of DMI significantly improved recovery after 7 days of DSS treatment and significantly reduced inflammatory gene expression in the colon.

Conclusion: Our data suggest that Acox1-produced itaconate has an important role in the regulation of inflammation during experimental colitis. Although myeloid cells have been thought to be major producers of Acox1 and itaconate, our data indicate that other cell types are involved. These results highlight the importance of this immunometabolic pathway and suggest that preservation or enhancement of this pathway with natural metabolites or metabolite derivatives could have beneficial effects during colitis.

P152
EVALUATING THE EFFICACY OF GLUTAMATE CARBOXYPEPTIDASE II (GCPII) INHIBITORS IN "WILD-TYPE" MICE: LESSONS LEARNED FROM THE DEDICATOR OF CYTOKINESIS 2 MUTANT MOUSE

Diane Peters, Lauren Norris, Barbara Slusher

Background: Glutamate carboxypeptidase II (GCPII) is highly upregulated in human IBD and colorectal cancer and is thought to be a therapeutic target for these diseases. We recently published that a spontaneously occurring loss-of-function mutation in dedicator of cytokinesis 2 (Dock2<sup>Hsd</sup>) that was present in commercially-purchased "wild-type" C57Bl/6NHsd mice increased their sensitivity to DSS-colitis and caused them to resemble murine IBD with respect to GCPII. The Dock2<sup>Hsd</sup> mice had significantly elevated colon GCPII activities and were sensitive to treatment with the GCPII inhibitor, 2-PMPA. We hypothesized that if colitis of the same severity were to be induced in Dock2<sup>WT</sup> mice, that they would also exhibit heightened colon GCPII activity and would be equally sensitive to 2-PMPA treatment.

Methods: DSS-colitis was induced in weight-, age- and gender-matched WT and Dock2<sup>Hsd</sup> mice (Dock2<sup>WT</sup> and Dock2<sup>Hsd</sup>). Increasing concentrations of DSS were utilized (2.5%-4.0%) and disease activity index was monitored daily. Results: With increased DSS concentrations (4%), a severe colitis could be established in the Dock2<sup>TTN</sup> mice which closely resembled the disease seen in Dock2<sup>TCH</sup> mice induced with 2.5% DSS. Interestingly, despite similarity in DAI scores and disease progression, the GCPII activity in colon of Dock2<sup>TCH</sup> mice (4% DSS) remained significantly lower than that of Dock2<sup>TCH</sup> mice (2.5% DSS) (p<0.001, t-test). Further, while 2-PMPA was effective in both groups, higher systemic doses were required in the IBD-resistant Dock2<sup>TCH</sup> mice.

Conclusions: Following identification that the spontaneously occurring mutation Dock2<sup>TCH</sup> influences murine DSS-colitis sensitivity and alters the activity of our therapeutic target protein, GCPII, in the colon, we sought to re-establish our DSS model using Dock2<sup>TCH</sup> mice. While we were successfully able to recapitulate disease severity in the Dock2<sup>TCH</sup> mice by increasing the DSS concentration from 2.5% to 4%, the underling disease biology was not conserved. Despite having comparable DAI scores at study termination, Dock2<sup>TCH</sup> mice had decreased GCPII activity in their colons relative to Dock2<sup>TCH</sup> mice and were less sensitive to inhibition with the GCPII inhibitor, 2-PMPA. These data caution that target protein expression must be verified even with subtle changes to experimental method when utilizing the DSS-colitis model.

P157
FBXO3-FBXL2 AXIS MODULATORS AS A NOVEL CLASS OF ORAL SMALL MOLECULE COMPOUNDS FOR THE TREATMENT OF CROHN’S DISEASE

Franca Angeli, Russell Wyborski, Bill Chen, Rama Mallampalli, Michael Lark

Background: Ubiquitination is a common post-translational modification, tagging proteins for degradation. The ubiquitin proteasome system is activated in Crohn’s Disease (CD), and modulation of its components might be a novel strategy for therapeutic intervention to control inflammation. The conjugation of ubiquitin to a target protein is orchestrated by a series of enzymatic reactions, the last step being catalyzed by a selective ubiquitin E3 ligase. Among ubiquitin E3 ligases, Fbx2 serves as a sentinel gatekeeper to limit inflammation by targeting and enhancing the degradation of tumor necrosis factor receptor associated factors (TRAF) proteins, which link cell surface signals (through NFκB signaling) to cytokine secretion. In addition, Fbx2 controls the ubiquitination of the inflammation, NOD-like receptor protein 3 (NLRC3), which mediates the release of interleukin (IL)1β and IL18 (Fig.1). Fbx2 protein itself is ubiquitinated and degraded by another protein, called Fbx3. Inhibition of Fbx3 results in increased Fbx2 levels and decreased TRAF and NLRC3. Individuals with a natural occurring polymorphism within Fbxo3<sup>B</sup>-<sup>DS</sup> have decreased lipopolysaccharide(LPS)-induced cytokine (TNFα, IL1β & IL6) production, Fbx3 and TRAF levels and increased Fbx2, providing human genetic target validation for Fbx3-Fbx2 axis modulation in inflammatory conditions. Dock2<sup>Hsd</sup> mice have a natural occurring polymorphism within Fbxo3<sup>B</sup>-<sup>DS</sup> and increase in colon W/L ratio; both were reversed by MET642 and anti-IL-12p40. MONOCHELIC CELLS. IN AN ACUTE DSS-MOUSE MODEL, ADMINISTRATION OF BC-1261 VIA DRINKING WATER AD LIBITUM (30 μg/ml) OR BY DAILY INTRAPEPTONEAL (IP) INJECTION (150 μg) FOR 5 DAYS RESULTED IN THE ATTENUATION OF THE SHORTENING OF COLONIC LENGTH, TISSUE DAMAGE (Fig.2A), AND TNFα AND IL6 TISSUE LEVELS (Fig.2B). IN A REPEATED DSS-MOUSE MODEL, BC-1261 (10 mg/kg bid) GIVEN ORALLY FOR 19 DAYS WAS COMPARABLE TO THE POSITIVE CONTROL (anti-p40) IN THE DAI COMPOSITE (Fig2.C), STOOL CONSISTENCY (Fig2.D), AND HISTOPATHOLOGY SCORE IN THE PROXIMAL COLON (Fig2.E). BC-1261 ALSO LED TO A SIGNIFICANTLY REDUCTION IN ENDOGENOUS SCORING (Fig2.F).

Conclusion: BC-1261, a Fbxo3 inhibitor, was efficacious in both acute and chronic preclinical models of colitis. Taken together, these results suggest the potential utility of selective Fbxo3-Fbx2 modulation in the treatment of CD.

P153
MET642, AN ORAL FXR AGONIST, IMPROVES COLITIS INDUCED BY ADOPTIVE T-CELL TRANSFER

Xueqing Liu, Robert O’Connell, Allison Bendele, Melissa Walker, Connor Osthen, Steve Govik, Johnny Nagasawa, Karena Douglas, Angelica Mikl, Nihin Lu, Jing Qian, Alvaro Ortiz, Pauline Chai, Douglas Zook, Kyoung-Jin Lee, Nicholas Smith, Brandee Wagner, Ken Song

Introduction: Farnesoid X receptor (FXR) is a ligand-activated nuclear hormone receptor expressed in the gastrointestinal tract, with high expression levels in the intestinal epithelium. Previously we have shown that FXR agonists with sustained activity decreased colitis in multiple chronic IBD models (i.e. Adoptive transfer colitis, Md1<sup>+-/-</sup> colitis, and SAMP1/YitFc ileitis). Because FXR agonists are dosed orally and do not have systemic immunosuppressive properties, they represent a novel and differentiated therapeutic approach for IBD. MET642 is an optimized, potent non-bile acid FXR agonist with sustained FXR activation. In this study, we examined the efficacy of MET642 in therapeutic treatment mode in adoptive T-cell transfer model.

Methods: Colonies were induced by transplanting CD4+CD45RBhi T-cells to recipient C.B-17 SCID mice. Body weights were monitored throughout study duration. MET642 in therapeutic treatment mode in adoptive T-cell transfer model. MET642, AN ORAL FXR AGONIST, IMPROVES COLITIS INDUCED BY ADOPTIVE T-CELL TRANSFER

Figure 1. Mechanism and Impact of Fbox3-Fbx2 Axis Modulation

Figure 2. BC-1261 is Effective in Mouse Models of Colitis