O-028
Use of CEACAMS Small Peptides as Immunomodulators in Crohn’s Disease
Rodra Giuliacci,1,2 Hovhannisyan Zarub2,3, Colombel Jean-Frédéric1,3, Yeretzian Garabedian2,3, Mayer Lloyd1,3
1Icahn School of Medicine at Mount Sinai, New York, New York; 2Icahn School of Medicine at Mount Sinai, New York, New York; 3Icahn School of Medicine at Mount Sinai, New York, New York

BACKGROUND: CD8+ regulatory T (T reg) cells suppressive function is impaired in Crohn’s disease (CD) patients due to a defect in CEACAMS expression on intestinal epithelial cells (IECs). We previously provided strong evidence of an immunoregulatory role of CEACAMS in acquisition of suppressive properties by peripheral blood (PB) CD8+ T cells upon their activation by CEACAMS. The aim of this study is to dissect the immunoregulatory region of CEACAMS to evaluate if it can be used to restore the impaired suppressive activity of CD8+ T cells in CD.

METHODS: Peptides of 5–10 amino acids were selected using a CEACAMS N-domain overlapping peptides library based on their ability to activate CD8+ T cells by inducing phosphorylation of PB CD8α associated Lck kinase and to induce the suppressive phenotype in PB CD8+ T cells measuring CD4+ T cell proliferation in co-cultures with activated-CD8+ T cells. To test the role of peptides with immunoregulatory activity in CD, we measured inhibition of CFSE-CD4+ T cell proliferation by CD LP CD8+ T cells upon activation with CEACAMS N-domain peptide and small CEACAMS peptides with immunoregulatory function.

RESULTS: We identified few peptides mapping near the sugar bridge (N70, 81) able to induce a suppressive phenotype on PB CD8+ T cells from healthy subjects. CEACAMS appeared to strengthen the suppressive properties of LP CD8+ T cells from healthy donors and to restore the impaired suppressive phenotype of CD LP CD8+ T cells as well as the few peptides tested in PB CD8+ T cells.

CONCLUSIONS: Our data strongly indicate that CEACAMS restores the suppressive phenotype of LP T reg in CD patients.

O-029
Chemical Chaperones Improve Intestinal Epithelial Cell Function and Ameliorate Symptoms of Inflammatory Bowel Disease
Wang Miao, Cao Stewart, Guerrero Gabriel, Kaufman Randall

Sanford-Burnham Medical Research Institute, La Jolla, California

BACKGROUND: The endoplasmic reticulum (ER) is the organelle in eukaryotic cells responsible for intracellular Ca2+ homeostasis, lipid biosynthesis and ER protein folding, transport and quality control. Alterations in the ER microenvironment disrupt ER protein folding to cause accumulation of unfolded/misfolded proteins, a condition termed ER stress, which activates the unfolded protein response (UPR). The UPR comprises 3 parallel signaling branches: PKR-like ER kinase (PERK)-mediated phosphorylation of eukaryotic translation initiation factor 2α (eIF2α), Inositol Requiring Enzyme 1-mediated X-box Binding Protein 1 mRNA splicing and Activating Transcription Factor 4 (ATF4) processing. The outcome of UPR activation involves transient attenuation of protein synthesis and transcriptional activation to increase capacity for ER protein folding, trafficking and secretion, and to increase protein degradative pathways. If these adaptive mechanisms cannot resolve the protein-folding defect, cells enter apoptosis. Recent studies showed that ER stress in intestinal epithelial cells (IECs) plays a fundamental role in inflammatory bowel disease (IBD) development. GWAS studies have also linked gene mutations in the UPR signaling pathway with IBD in humans, suggesting the significance of ER stress and UPR in the etiology of IBD. Therefore we hypothesize that restoration of ER homeostasis will improve IEC function and ameliorate IBD.

METHODS: We first examined the response to dextran sodium sulfate (DSS) or Salmonella infection in mice with IEC-specific Ser51Ala mutation at the PERK phosphorylation site in eIF2α or with deletion in Atf6α. To restore ER homeostasis, we treated IBD mouse models, including mice fed DSS to induce acute or chronic colitis (models of barrier dysfunction), Il10−/− mice (model of colitis) and TnfαLk−/− (model of Crohn’s ileitis), with the chemical chaperones, 4-phenylbutyric acid (PBA) or tauroursodeoxycholate (TUDCA). Both TUDCA and PBA are FDA-approved bioactive small molecules that function to facilitate protein folding and reduce ER stress both in vitro and in vivo by stabilizing protein-folding intermediates and preventing protein aggregation. Bodyweight and rectal bleeding were monitored daily. Colon tissue were harvested for histopathology, and IECs were isolated for Western blot and RT-PCR. In addition, immunohistochemistry (IHC) staining for ER stress was performed on biopsy samples from IBD patients and healthy controls.

RESULTS: We found that eIF2α phosphorylation is required to protect IEC function when challenged with DSS or Salmonella infection. Furthermore, Atf6α null mice which display significantly reduced expression of many ER chaperones (BIP, Grp94 and PDI) and hyperactivation of pro-apoptotic UPR signaling in colon IECs, are more sensitive to DSS than control mice. Specifically, we found that ER stress caused by deletion of the ER co-chaperone gene PSB4/Dnajc3 excrates experimential colitis in mice. Indeed, IECs in IBD patients experienced more severe ER stress than the controls, judged by BIP IHC staining in intestinal biopsy samples. In contrast, oral delivery of PBA or TUDCA dramatically decreases the clinical, histological and biochemical signs of inflammation in all tested IBD mouse models through reducing ER stress signaling in IECs.

CONCLUSIONS: These findings indicate that ER stress plays a fundamental role in IBD development and encourage the clinical evaluation of PBA or TUDCA administration as adjuvant therapy for IBD patients.

O-030
Colon Epithelial EGFR Inhibits NFκB-Dependent Innate Immune Responses Through CCDC50
Dubé Philip, Girish Nandini, Polk D

Children’s Hospital Los Angeles, Los Angeles, California

BACKGROUND: Epidermal growth factor receptor (EGFR) is a key regulator of epithelial proliferation, survival and migration in the colon, where it functions to stimulate epithelial repair following injury. However, recent work by our lab has uncovered a novel role for epithelial EGFR to limit inflammation and subsequent tumorigenesis in mouse colitis models. This anti-inflammatory role for EGFR suggested that inhibition of epithelial innate immune responses might be a key mechanism underlying the therapeutic potential for EG in IBD. In this study, our aim was to determine how EGFR regulated the epithelial cell response to lipopolysaccharide (LPS), and how a novel target of the EGFR kinase, coiled-coil domain containing 50 (CCDC50), mediates this response. Hypothesis: EGFR inhibits colon epithelial responses to LPS by blocking NFκB activation through the adapter protein CCDC50.

METHODS: YAMC mouse colon epithelial (MCE) cells or human HT-29 cells were stimulated with LPS or with and without EGFR co-treatment. NFκB activity was measured in YAMC cells (NF-YAMC) stably transfected with an NFκB luciferase reporter. IL-8 and CXCL2 mRNA were measured by qPCR, and CXCL2 secretion was measured by ELISA. CCDC50 or TNFαIP3 were knocked down by siRNA with non-targeting siRNA as control. Knockdown was confirmed by qPCR.

RESULTS: LPS-induced concentration-dependent NFκB activity (EC50 = 40 ng/mL) in NF-YAMC cells, which was inhibited with escalating concentrations of EG (see figure; IC50 = 0.2 ng/mL EG, n = 6). This inhibitory effect of EG was blocked with an EGFR inhibitor, and in MCE cells expressing a dominant-negative EGFR (EGFΔa2). EG alone did not affect basal NFκB activity. This response was not specific for LPS, as EG also inhibited NFκB activity induced by L18-MDP (a cell-permeable muramyl dipeptide analog). We next determined that EGF inhibited epithelial chemokine production following LPS stimulation. EG inhibited LPS-stimulated induction of mRNA for IL-8 (HT-29 cells) and CXCL2 (YAMC and HT-29 cells), and protein for CXCL2 (YAMC cells). Using immunoprecipitation and Western blot, we determined that EGFR induced tyrosine phosphorylation of CCDC50 in YAMC cells. Furthermore, siRNA-mediated CCDC50 knockdown blocked the ability of EG to inhibit LPS-induced NFκB activity and CXCL2 mRNA expression, compared to non-targeting siRNA control. Since CCDC50 has previously shown to interact with TNFαIP3 (A20), we determined whether CCDC50 required TNFαIP3 to inhibit LPS-induced NFκB activity by siRNA-mediating TNFαIP3 knockdown. However, loss of TNFαIP3 did not prevent the inhibitory effect of EGFR, suggesting that the EGFR/CCDC50 mechanism involves an alternate pathway for NFκB inhibition.

CONCLUSIONS: The inhibition of LPS-induced NFκB signaling by EGFR activation in the colonic epithelium represents a novel mechanism through which EGFR restrains inflammatory responses. EGFR inhibits NFκB through a novel adapter protein, CCDC50, which functions independently of TNFαIP3, and current work is underway to determine this mechanism. This study uncovers a previously underappreciated protective mechanism for EGFR to inhibit innate immune responses in the colon by blunting the response of colon epithelial cells to microbial triggers, and thus limiting subsequent chemokine production and inflammation. Thus, EGFR and CCDC50 are promising targets to moduclate mucosal inflammation in IBD.