

to clear the infection, we hypothesized that guar gum fed mice might clear *CR* infection better than controls. To our surprise, guar gum fed WT mice shed higher numbers of *CR* in the feces than the cellulose group. The guar gum fed mice had lower body weights, colomegaly, an elevated level of colonic and serum Lcn2, and higher serum SAA than the control group. Collectively, guar gum failed to protect against *CR*-induced colonic pathology. Altogether, the work demonstrates that guar gum feeding may exacerbate colonic inflammation following immune-hyperactivation, chemical, and infectious injury. Cautioning IBD patients to monitor their consumption of guar gum fiber might be a way to reduce the severity of intestinal inflammation.

DYSREGULATION OF GASTROINTESTINAL RAGE (RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS) EXPRESSION IN A SPONTANEOUS ANIMAL MODEL OF INFLAMMATORY BOWEL DISEASE

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The receptor for advanced glycation end products (RAGE), a pattern recognition receptor, plays a role in chronic inflammation. Abrogation of proinflammatory RAGE signaling by ligand binding (e.g., S100/calgranulins) to soluble RAGE decoy (sRAGE) is a promising novel therapeutic avenue for chronic inflammatory diseases, such as inflammatory bowel disease (IBD). However, the opportunities for studying S100/calgranulin-RAGE pathways in conventional animal models are limited due to species differences in the expression and function of S100/calgranulins (e.g., lack of the S100A12 protein in rodents). The pathogenesis of IBD in dogs involves dysregulated innate immune responses, and serum sRAGE levels are decreased in canine IBD and normalize only with clinical remission. Thus, canine IBD may serve as a spontaneous model of IBD for such studies. This study evaluated gastrointestinal mucosal RAGE expression in dogs with IBD and the binding of RAGE to canine S100/calgranulin ligands.

Epithelial RAGE expression was quantified in endoscopic gastrointestinal (i.e., gastric, duodenal, ileal, and colonic) biopsies from 12 dogs with IBD and 9 healthy control dogs by laser scanning microscopy. RAGE expression was compared between both groups of dogs and was tested for an association with patient characteristics, clinical variables, histologic lesion severity, and biomarkers of extra-gastrointestinal disease, systemic or gastrointestinal inflammation, function, or protein loss. Statistical significance (non-parametric tests) was set at $p < 0.05$. RAGE:S100/calgranulin binding was investigated by immunoassay and electrophoretic techniques.

RAGE expression was detected in all biopsies evaluated. Epithelial RAGE expression in the duodenum and colon was significantly higher in dogs with IBD than in healthy controls ($p < 0.04$), with a trend for overexpression in the ileum, underexpression in the stomach, and a general shift towards more basal than apical epithelial RAGE expression. Serum sRAGE was correlated with duodenal RAGE expression. Several histologic (structural and inflammatory) lesion criteria and markers of gastrointestinal inflammation or protein loss were related to segmental RAGE expression (all $p < 0.04$). *In vitro*, canine RAGE:S100A12 binding appeared more pronounced than RAGE:S100A8/A9 binding.

Alterations in the epithelial expression of RAGE along the gastrointestinal tract provide evidence for a dysregulated sRAGE/RAGE axis as a characteristic of canine IBD. S100/calgranulin (S100A8/A9 and S100A12) proteins are ligands for RAGE in dogs. The role of RAGE signaling in IBD pathogenesis and its potential for developing novel targeted therapeutics warrants further exploration. Furthermore, canine IBD is a suitable spontaneous model for human IBD that may benefit further research into pathway-specific IBD treatment options that target the sRAGE/RAGE axis.

MUC1-C IS A DRUGGABLE TARGET FOR TREATMENT OF COLITIS AND PROGRESSION OF COLITIS-ASSOCIATED COLORECTAL CANCER

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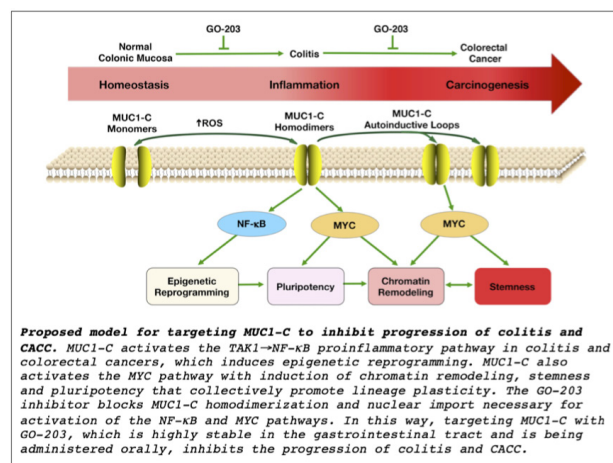
The MUC1-C oncoprotein evolved in mammals to protect epithelial cells, such as those lining the gastrointestinal tract, from loss of homeostasis. In this way, MUC1-C activates pathways that contribute to inflammation, proliferation and remodeling associated with the wound healing response.

MUC1-C is upregulated in human tissues from inflamed ulcerative colitis (UC) mucosa as compared to that from normal and uninfamed UC mucosa. MUC1-C is also upregulated in a mouse MUC1^{+/+}/IL-10^{-/-} model of colitis, consistent with its involvement in the inflammatory response. MUC1-C forms a direct complex with NF- κ B p65 and promotes the activation of NF- κ B target genes, including MUC1 itself in an auto-inductive circuit. MUC1-C thereby drives proinflammatory NF- κ B pathway genes in human inflamed UC tissues and in the genetically engineered mouse model (GEMM) of colitis. Mechanistically, MUC1-C induces the TGF- β activated kinase 1 (TAK1), which is an essential effector of proinflammatory NF- κ B signaling. Of further significance, MUC1-C drives the TAK1 \rightarrow NF- κ B p65 pathway in human colon cancer cell lines, and MUC1 and TAK1 are upregulated in human

colon cancers. These seminal findings supported the notion that MUC1-C contributes to colitis and progression to colon cancer.

To extend these studies, MUC1-C was targeted with an inhibitor that blocks its homodimerization and function. Remarkably, treatment of the MUC1^{+/+}/IL-10^{-/-} GEMM with the GO-203 inhibitor was associated with decreases in the severity of colitis and progression of colitis to dysplasia and carcinomas. Intestinal stem cells (ISCs) that express Lgr5 are of importance in the inflammatory response to colitis and in progression to colitis-associated colorectal cancer (CACC). Targeting MUC1-C with GO-203 in mouse models of colitis suppressed Lgr5 expression, as well as induction of MYC and other core pluripotency factors. By extension to human colon cancer cells, we found that MUC1-C drives MYC with activation of LGR5 and stemness. MUC1-C also induces cancer stem cell (CSC) markers (BMI1, ALDH1, FOXA1, LIN28B) and the OCT4, SOX2, and NANOG pluripotency factors. Consistent with driving the CSC state, targeting MUC1-C suppressed the capacity of CRC cells to promote wound healing, invasion, self-renewal, and tumorigenicity. Analysis of human tissues further demonstrated that MUC1 expression associates with activation of inflammatory pathways, development of colitis, and aggressiveness of CRCs.

These results collectively indicate that MUC1-C is of importance for integrating stemness and pluripotency in colitis and CRC. Of clinical relevance, the findings further indicate that MUC1-C represents a previously unrecognized target that is potentially druggable with orally administered GO-203 now being tested in the GEMMs for treating progression of colitis and CRC.



NANOPARTICLE-ENCAPSULATED BROMODOMAIN-CONTAINING PROTEIN 4 INHIBITORS FOR THERAPEUTICS OF INFLAMMATORY BOWEL DISEASE

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Ulcerative Colitis (UC) and Crohn's disease (CD), the two major types of inflammatory bowel disease (IBD), are chronic diseases with recurrent symptoms and significant morbidity. Long-term persistence of chronic inflammation in IBD is among the major factors contributing to neoplastic transformation and the development of colitis-associated colorectal cancer. There exists a lack of efficient medications for IBD, primarily due to either limited efficacy or side effects. Targeting bromodomain-containing protein 4 (BRD4) represents a novel therapeutic strategy for IBD. Recently, we have successfully identified proprietary, highly potent, and specific BRD4 inhibitors which significantly suppressed the initiation of mucosal inflammation and chronicity in proof-of-concept studies in several animal models of IBD.

One of the most pressing challenges in current IBD research is to develop technologies to enable mucosal targeted drug delivery systems that enhance efficacy and decrease side effects. Intriguingly, we found that local delivery of our first-generation BRD4 inhibitors encapsulated in nanoparticles can achieve significantly higher *in vivo* efficacy at much lower doses than that attained by systemic administration. Local delivery of nanoparticle-encapsulated BRD4 inhibitors by designing nanoparticles that bind to inflamed epithelium would offer superior pharmacotherapy for IBD with higher efficacy, specific delivery, long-lasting release, and a better therapeutic and safety window. We have successfully encapsulated our BRD4 inhibitors in biodegradable nanoparticles with excellent encapsulation efficiency and favorable particle size. Our new generation of BRD4 inhibitors ZL0513, ZL0742, and ZL0591 were encapsulated by a modified solvent displacement method using a polymeric matrix of PEGylated poly (lactic-co-glycolic acid) (PLGA). To evaluate their potential cytotoxic effects, we incubated human colonic

epithelial cells (HCECs) and peripheral blood mononuclear cells (PBMCs) with PEGylated-PLGA-ZL0513, ZL0591, and ZL0742 overnight. No apparent increase in cell death was detected in HCECs or PBMCs even at 40 μ M. Furthermore, oral administration of our nano-encapsulated BRD4 inhibitors at the dosage of 2 mg/kg effectively block colonic inflammation in both IBD animal models of dextran sulfate sodium (DSS)-induced colitis and oxazolone (OXA)-induced colitis.

Collectively, our compelling *in vivo* efficacy data support that our nano-encapsulated BRD4 inhibitors effectively block colonic inflammation in animal models of IBD. Local delivery of nanoparticle-encapsulated BRD4 inhibitors may offer superior pharmacotherapy for IBD patients.

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STOCHASTIC INTERINDIVIDUAL MICROBIOME VARIATION MAY GUIDE PROTECTIVE PERINATAL PROBIOTIC DEVELOPMENT AGAINST IBD

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The purpose of our experiment was to explore how stochastic inter-individual variation in the mammalian gut microbiome may link to inflammatory bowel disease (IBD) susceptibility, and guide the development of a perinatal preventative probiotic.

Dextran sodium sulfate (DSS) was introduced to C57BL/6J mice to induce acute colitis as a model of IBD. Potentially protective bacteria were identified using a discovery-validation cohort approach towards stochastic DSS susceptibility. *Lactobacilli* (two different cocktails of *L. reuteri* and *L. johnsonii* strains) or control media were supplemented by mouth to dams prior to delivery and during lactation (i.e. perinatal probiotic). The pups were evaluated for DSS susceptibility at young adulthood.

Fecal *Lactobacillus* was increased in the DSS-resistant mice in both the discovery and validation cohorts. Maternal supplementation of female offspring with an *L. reuteri* cocktail (strains 6798-1, 6798-jm, and 6798-cm) induced progressive microbiome separation and protection against colitis by young adulthood.

Maternal supplementation of *L. reuteri* could confer protection against DSS colitis in young adult female mice. This work is the first to exploit stochastic mammalian microbiome variation to guide microbial therapeutic identification. Our findings underscore neonatal microbiome plasticity and set the stage for the potential development of perinatally deliverable protective probiotics against human IBD.

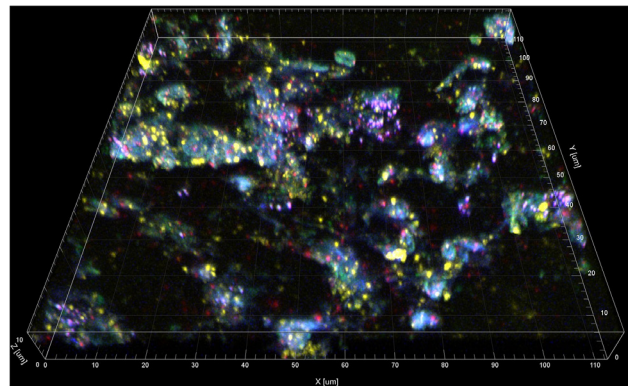
THE ROLE OF MYELOPEROXIDASE AND NEUTROPHIL EXTRACELLULAR TRAPS IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

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Neutrophils are short-lived immune cells that represent the major cell type recruited to the inflamed bowel releasing their azurophilic granules containing enzymes myeloperoxidase (MPO). Fecal and serum MPO levels has previously been shown to correlate to disease severity in IBD patients. MPO, in the presence of H₂O₂ and free Cl⁻ undergoes a halogenation cycle, yielding the two-electron oxidant, hypochlorous acid (HOCl) - a potent bactericidal agent. However, chronic intestinal exposure to MPO/HOCl due to perpetual inflammation may cause secondary host-tissue injury and cell death. Neutrophil Extracellular Trap (NET)osis is a specialised form of neutrophil death where MPO is entrapped in a DNA scaffold and continues to elicit HOCl activity and may further contribute to host-tissue injury.

We investigated the presence of NETs in surgically excised ileum samples from CD and healthy patients using advanced confocal microscopic techniques and found MPO, Neutrophil Elastase (NE) and Citrullinated Histone h3 (CitH3) - critical components of NET formation, individually positively correlate to the severity of histopathological intestinal injury. Furthermore, multiplex Opal™ IHC performed using LMS880 Airyscan-modulated microscopy with z-stacking revealed colocalization of NE, MPO, CitH3 and DAPI indicating the extensive presence of NETs in severely affected CD tissue. Using two pharmacological inhibitors of MPO in a dextran sodium sulphate (DSS) model of murine colitis, we demonstrated the pathological role of MPO in experimental colitis. MPO inhibitors, TEMPOL and AZD3241 delivered via daily *i.p.* significantly rescued the course of colitis by abrogating clinical indices including body weight loss, disease activity index, inhibiting serum peroxidation, and preserving colon length, while significantly mitigating histoarchitectural damage associated with DSS-induced colitis. We also showed that MPO inhibition decreased neutrophil migration to the gut, suggesting MPO may play a role in perpetuating the inflammatory cell by further recruiting cells to the inflamed gut.

Collectively, we have shown for the first time that MPO is not only an important clinical marker of disease severity but may also play a critical role in perpetuating host-tissue damage and inflammation.



TOXICOLOGICAL FINDINGS OF A RECOMBINANT CHOLERA TOXIN B SUBUNIT VARIANT WITH THERAPEUTIC POTENTIAL IN ULCERATIVE COLITIS

Micaela Reeves, Daniel Tuse, Krystal Hamorsky, Joshua Royal, Nobuyuki Matoba

Background: The cholera toxin B subunit (CTB) is the nontoxic and homopentameric component of the holotoxin. Upon binding to GM1 ganglioside on the surface of epithelial cells, CTB mediates entry and retrograde transport through the endomembrane system and disengages the catalytic A subunit in the endoplasmic reticulum (ER). EPICERTIN (EPT) is a recombinant variant of CTB with a non-native C-terminal extension harboring an ER-retention motif, KDEL. We have found that increased ER-retention time resulting from this modification allowed EPT to induce an unfolded protein response and TGF- β signaling in colon epithelial cells, triggering wound healing activity in preclinical colitis models. The unique epithelial repair activity of EPT hints at its therapeutic potential in ulcerative colitis.

Objective: We aim to develop data supporting a first-in-human clinical trial with an EPT enema indicated for ulcerative colitis. Here, we evaluated the efficacy and toxicity of intrarectal (IR) administration of EPT in preclinical rodent models.

Results: IR administration of EPT at 0.1 and 1 μ M to female C57/BL6 mice (0.6 and 6.1 μ g/animal) with acute dextran sodium sulfate (DSS)-induced colitis resulted in decreased disease activity index scores and increased body weight recovery, supporting a target therapeutic dose of ≤ 1 μ M for clinical administration. A dose-escalation study was performed following a single IR exposure at 1, 2 and 5 μ M (61.4, 122.8 and 307 μ g/animal) in male and female Sprague Dawley rats. No drug-related adverse effects were evident in clinical observations, including clinical pathology and gross necropsy, even at the highest dose tested. A pharmacokinetics study was performed in male and female mice dosed with a 1 or 10 μ M (6.1 and 61.4 μ g/animal) IV bolus of EPT. Plasma samples were collected periodically for up to 24 h postdose. EPT concentrations were highest at first collection and decreased steadily until unquantifiable by 4 h. The elimination phase half-life was 0.26 to 0.3 h. When healthy and DSS-induced colitic mice (n = 72) were dosed with 1 or 10 μ M EPT IR, marginal amounts of EPT were found in only 4 plasma samples scattered across groups and time points, suggesting that systemic exposure after IR administration is negligible.

Conclusion: These data support further development of EPT as a potential therapeutic for ulcerative colitis.

Comparative Effectiveness Studies

COMPARATIVE EFFECTIVENESS AND SAFETY OF VEDOLIZUMAB AND ANTI-TUMOR NECROSIS FACTOR AGENTS IN OLDER ADULTS WITH INFLAMMATORY BOWEL DISEASES IN MEDICARE ADMINISTRATIVE CLAIMS DATABASE

Bharati Kochar, Virginia Pate, Michael Kappelman, Millie Long, Ashwin Ananthakrishnan, Andrew Chan, Robert Sandler

Background: The number of older adults with inflammatory bowel diseases (IBD) is increasing. Older adults with IBD are less likely to receive effective immunosuppression. We aimed to determine efficacy and safety of biologic therapies in older adults with IBD.

Methods: We conducted a retrospective cohort study using an active comparator, new user design in a 20% random sample from the 50 state Medicare claims