

*A Murine Colitis Molecular Inflammatory Signature Causes
a Serum Triggered Inhibition of Colonic Circular Smooth Muscle*

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Abstract:

Background. Ulcerative Colitis (UC) is characterized as an inflammatory bowel disease, currently impacting 1.5 million people in the United States. Symptoms are caused by an autoimmune mediated inflammation resulting in ulcerations within the mucosal lining of the colon. UC presents with fatigue, bloody stools, diarrhea, abdominal pain, reduced appetite, and weight loss. In addition, UC patients suffer from unexplained debilitating intestinal motility abnormalities. Management is mediated with pharmacotherapy and surgery, but there is currently no cure for UC.

Objective. Our objective was to investigate a novel hypothesis that the molecular inflammatory responses of colitis cause changes in colonic motility through the inflammatory induction of a transmural colonic microvascular leak, which disruptively triggers enteric motor neuron activity.

Methods. C57Bl/6 were fed water or dextran sodium sulfate (DSS) 3% until sacrifice on day 10. Colons were grossly and histologically inspected (N=5,5) and mucosal barrier failure tested by orally fed 0.4 μ m microspheres (N=4,4). Spatial distribution of neutrophils, F4/80⁺ infiltrates & microvascular leak of IV 0.4 μ m microspheres were quantified using a novel lamina propria

(LP)/muscularis externa (ME) whole-mount (N=6,6). Colonic tissue and serum cytokines were determined using a MAGPIX cytokine panel (N=6,6). Organ bath colonic circular and longitudinal muscle strip contractility responses to serum (0.5-5%) in Krebs, LNA (30 μ M) or LNA+atropine (3 μ M) were quantified (N=4 each) (p-values <0.05 between control and DSS = significance).

Results. At sacrifice, colonic DSS histology exhibited hemorrhagic mucosal sloughing with mucosal barrier failure demonstrated by the copious presence of 0.4 μ m fluorescent microspheres within the LP and ME after oral administration. No microsphere transference occurred in controls. Analysis of neutrophils and F4/80⁺ infiltrates within the LP demonstrated DSS-induced dense infiltrates that were clustered around the LP venules (cntrl=162.0 \pm 6.01 vs. DSS=48.4 \pm 8.05 luminosity, 100X field). DSS colonic ME whole-mounts had a mild significant increased presence of neutrophils compared to controls (cntrl=1.5 \pm 0.97 vs. DSS=24.2 \pm 1.3, 100X field). Immune analysis of the conditioned media of the organ cultured LP/ME tissue demonstrated significant increases in VEGF, IL-10, MIP-1 α & GM-CSF compared to control. Serum cytokines also showed a systemic inflammatory response (VEGF, IL-6 & MCP-1). Colitis caused a profuse vascular leak of IV administered 0.4 μ m microspheres within both the LP and ME. Exposure of colonic circular muscles to serum 2.5% caused a remarkable 88 \pm 7.8% decrease in spontaneous contractions, which was significantly blocked by the neural nitric oxide blocker LNA revealing a large partially atropine sensitive excitatory contractile response. Threshold responses occurred at 0.5% serum.

Conclusion. The data demonstrate that colitis inflammatory mediators (VEGF) can induce a significant microvascular leak within the lamina propria and muscularis externa. And, that serum potentially activates nitrergic and cholinergic motor neurons, which dramatically alters gastrointestinal motor responses. The blockade of these serum-induced myenteric motor responses may be a novel target to ameliorate the debilitating motility abnormalities of IBD.

Christian world view integration:

Physicians have a unique opportunity to impact thousands of patients throughout their career by incorporating a Christian world view in their practice on a daily basis. Obtaining a medical degree from Liberty University College of Osteopathic Medicine, provides a platform to share how a Christian worldview has impacted our current educational experience, while providing a foundation for our future careers as physicians. Conducting research at LUCOM allows our team to work in a cohesive environment, surrounded by staff, administration, and fellow classmates who share a Christian worldview, that we believe facilitates advancements in our field. We place significant importance on a servant's heart and showing the love of Christ through our dedication to others. As student researchers, we are humbled to study an area of medicine that affects millions of people in today's society. The countless hours of work to formulate hypothesis and conduct experiments to determine conclusions about unknown aspects of diseases emulates humility. Our goal is to advance medicine, specifically in gastroenterology, and ultimately help others who are struggling with UC.