

Second Annual Progress Report to the CCFC – June 2009

Grant Title: Goblet Cell Mediators and their Impact on Mucosal Protection and Susceptibility to Colitis.

Applicants: Dr. Bruce Vallance (principal), Dr. Kris Chadee

Original Lay Summary from 2006 application

Inflammatory Bowel Disease (IBD) is believed to occur in genetically predisposed individuals when their immune systems undergo prolonged exposure to intestinal bacteria. For this to occur, we hypothesize that intestinal inflammation causes the disruption of protective barriers within the gastrointestinal tract, allowing bacteria to leak across the epithelium and activate immune cells. The intestinal mucus layer is one such barrier, and while goblet cells and the mucus they produce are both depleted during IBD, the mechanisms underlying their depletion are unclear. Using a bacterial induced model of colitis in mice, we found that inflammation dramatically reduces mucus production by goblet cells, but increases their production of a novel protein called RELM- β . Our studies suggest these changes in goblet cell function subsequently worsen colitis in two ways. The reduction in colonic mucus impairs intestinal protection, permitting dangerous bacteria to adhere to and translocate across the intestinal epithelium, increasing inflammation. In addition, mice that express RELM- β undergo more severe inflammation and tissue damage than mice lacking this protein, suggesting that RELM- β has pro-inflammatory actions within the colon. Our proposed studies will explore in greater detail how inflammation induced changes in goblet cell function aggravate and perpetuate colitis, identifying potential new therapeutic targets in IBD.

Technical Update

The etiology of Inflammatory Bowel Diseases (IBD) is unclear however the exaggerated and maladaptive inflammatory responses that accompany these diseases are thought to develop in genetically predisposed individuals following abnormal exposure of their immune systems to enteric bacteria. For this exposure to occur, the intestinal barriers that normally prevent luminal bacteria from contacting the immune system appear to be impaired in IBD patients. There are two major mucosal barriers found within the GI tract, the first being the epithelial layer that functions by forming tight junctions between epithelial cells. The other major protective mucosal barrier is provided by goblet cells and the mucus layer they create that overlies the intestinal epithelium. Interestingly, in concert with barrier changes, IBD is also associated with distinct abnormalities in the makeup of gut bacteria. For example, the intestines of IBD patients carry unusual types of bacteria that either loosely adhere to the intestinal surface or escape from the intestinal lumen and invade deeper tissues where they cause inflammation. Our specific goals this year were to explore the role that the mucus layer plays in protecting the intestine against bacteria, and develop a better understanding of whether the mucus layer contributes to the unusual nature of the microbial populations found in IBD patients.

A frequent observation in inflamed IBD tissues is a dramatic reduction in goblet cell numbers and a thinner mucus layer as assessed by histological staining for goblet cell mucins. This pathology is termed “goblet cell depletion” and our experiments over the first year of this grant (2007-2008) determined that the goblet cell depletion that occurs in mice during infection by the bacterial pathogen *Citrobacter rodentium* requires the actions of T lymphocytes. The immune system appears to act by increasing goblet cell turnover during infection, limiting the maturity of these cells and their production of mucins, with the result that mucin filled goblet cells disappear from histological sections of the colon.

Over the last year (2008-2009), we have assessed what role the mucin gene *Muc2* plays in protecting the colon from bacteria. We have been testing mice deficient in *Muc2* and therefore lacking an intestinal mucus layer. We found these mice to be extremely susceptible to infection by the enteric bacterial pathogen *Citrobacter rodentium*, ultimately succumbing to infection within a few days. Assessment of bacterial counts found a 10-100 fold higher *C. rodentium* bacterial burden in the *Muc2* deficient mice, and strikingly, most of these bacteria were loosely adherent to the intestine, where they appeared to form biofilms. These biofilms were often found overlying severely damaged mucosa, suggesting that the bacteria were causing much of the damage. In these and other regions of the colon, bacteria were seen to be leaking out of the intestinal lumen and into deeper tissues where they were associated with mucosal inflammation. To explore the mechanisms by which *Muc2* protects the gut from bacteria, we studied *Muc2* release in wildtype mice. In collaboration with Dr. Kris Chadee's lab, we found that exposure to *C. rodentium*, either in culture or in vivo, led to an increase in *Muc2* release by goblet cells, potentially acting to "flush" the bacteria out of the colon. In the absence of this flushing, *C. rodentium* and perhaps other bacteria can more easily adhere to the intestinal surface, where they can damage the epithelial barrier and leak across causing colitis.

In summary, our results indicate that *Muc2* and the intestinal mucus layer helps to protect the intestine from pathogenic enteric bacteria by flushing these microbes out of the intestine, thereby preventing them from causing damage and inflammation in the GI tract. Defects in the expression or function of intestinal mucins may explain why the intestines of patients with IBD carry more adherent intestinal microbes than healthy intestines.

Lay Translation

There is significant evidence that Inflammatory Bowel Disease (IBD) occurs when the intestine becomes leaky, allowing bacteria to escape the inside of the intestine and reach immune cells, causing inflammation. Several studies have suggested that mucus, the sticky substance that coats our insides might play a role in preventing IBD, since it helps keep bacteria inside our intestine. We therefore tested mice that have mucus, as well as mice that lack mucus, by infecting them with bacteria that can cause a form of IBD. We found that mice that have mucus, expressed more mucus when they were exposed to the bacteria, and the mucus helped to wash away the bacteria. In contrast, mice lacking mucus carried 10-100 times more bacteria than other mice. In fact, in the absence of mucus, the bacteria formed massive clusters on the intestinal surface, causing severe damage to the intestine, and many of these bacteria escaped out of the intestine causing IBD. Based on these results, we believe that intestinal mucus plays a critical role, not only by preventing bacteria from escaping the intestines, but also by flushing them out of the intestine, therefore defects in mucus may be a key factor in causing IBD.

List of publications from or facilitated by this CCFC grant (2008-2009 only)

(underlined names are trainees under my supervision)

Gibson D.L., McNagny K.M., Ropelseki M., Bergstrom K.S.B., Mansson L., Montero M., Sham A., Ma C., Huang J.T., and **Vallance B.A.*** Mucosal integrity is protected by tissue resident TLR2 responses while injured by hematopoietic TLR4 responses during acute infectious colitis. Gastroenterology., in prep. * corresponding author

Bergstrom K.S.B., Kissoon-Singh V., Gibson D.L., Ma C., Montero M., Huang T., Sham A., Velcich A., Finlay B.B., Chadee K.,* and **Vallance B.A.*** Mucin-2 plays a critical role in host defense by preventing maladaptive bacterial-host interactions during infectious colitis. PLoS Pathogens., in prep. * co-corresponding authors

Mansson L.,# Montero M.,# Bergstrom K., Ma C., Huang J.T., Man C., Grassl G.A.,
and **Vallance B.A.*** MyD88-dependent fibroblast proliferation and
cyclooxygenase-2 expression underlie bacterial-driven intestinal fibrosis. J.
Immunol., submitted. # co-first authors, * corresponding author

Hollenbach E., Montero M., Neumann M., Ryz N., Vieth M., Zoeller M., Stremmel
W., Malfertheiner P., **Vallance B.A.**, and Ruhl A. A role for enteric glial cells in
innate and adaptive immune defense in the GI tract. Gut., submitted.

Wu X., Conlin V.S., Dai C., **Vallance B.A.**, Buchan A.M., Boyer L., and Jacobson
K. Vasoactive intestinal peptide ameliorates intestinal epithelial barrier disruption
associated with *Citrobacter rodentium*-induced colitis. Am. J. Physiol., accepted
with revisions.

Grassl G., Valdez Y., Bergstrom K.S.B., Gros P., Finlay B.B.*, and **Vallance B.A.***
Chronic Salmonella enteritis in resistant mice leads to severe intestinal fibrosis.
Gastroenterology., 134(3): 768-780, 2008. (cited 5 times) Featured in
Gastroenterology: 134(3): 872-875.

Bergstrom K.S.B., Guttman J.A., Rumi M., Ma C., Khan M.A., Vogl A.W., and
Vallance B.A.* Modulation of intestinal goblet cell function during infection by an
attaching/effacing bacterial pathogen. Infect. & Immun., 76(2): 796-811, 2008 *
corresponding author

Wu X., Vallance B.A., Boyer L., Walker J., Bergstrom K.S.B., Madsen K.L., O'Kusky
J., Buchan A.M., and Jacobson K.J. The probiotic yeast *Saccharomyces boulardii*
ameliorates *Citrobacter rodentium* induced colitis and associated epithelial barrier
dysfunction in infected mice. Am. J. Physiol., 294(1): G295-G306, 2008. (cited 9
times)

Oral presentations by Dr. Bruce Vallance

- 2009 (June 16th) Café Scientifique, Vancouver, British Columbia, Canada Title: *Knowing your gut in sickness and in health.*
- 2009 (June 4th) ORDCF/Farncombe Institute Workshop, Hamilton, Ontario, Canada Title: *The role of the microbiota in enteric infections.*
- 2009 (April 24th) IBD 2009, CCFC Sponsored Conference, Toronto, Ontario, Canada Title: *Using bacterial pathogens to explore inflammatory and tissue repair mechanisms during colitis.*
- 2009 (April 4th) IBD Family Day, BC Children's Hospital, Vancouver, British Columbia, Canada Title: *Progress at the Vancouver Gastrointestinal Disease Research Group*
- 2009 (March 1st) Canadian Digestive Diseases Week, Banff, Alberta, Canada Title: *How do commensal bacteria communicate with the host in health and disease?*
- 2009 (January 25th) GIRG Research Forum, Delta Kananaskis, Kananaskis, Alberta, Canada. Title: *Modeling Crohn's disease using bacterial pathogens.*

Supplementary funding

Not applicable

Personnel working on this CCFC grant

Since the trainees originally funded by this grant obtained personal awards, additional trainees were recruited to work on this project, which enabled the completion of additional studies, including the development of a mouse model of intestinal fibrosis.

Postdoctoral fellows

Dr. Marinieve Montero (funded by a CAG/CCFC fellowship)

Dr. Lisa Mansson (funded by a CAG/Astra Zeneca fellowship)

Graduate Students

Mr. Kirk Bergstrom (funded by CIHR and MSFHR senior studentships)

Mr. Andy Sham (funded by this grant)

Technicians

Ms. Caixia Ma (funded by this grant)