

## Disruptions of epithelial integrity in the pathogenesis of IBD:

### The effects of *Campylobacter jejuni*

- Interim report for year 2 -

#### 1. Technical summary

*Campylobacter* enteritis represents a risk factor for the development of symptoms in inflammatory bowel disease (IBD) via unknown mechanisms. As IBD patients exhibit inflammatory responses to their commensal intestinal microflora, factors that induce translocation of commensal bacteria across the intestinal epithelium may contribute to IBD pathogenesis. In turn, therapeutic strategies that would control these events may inhibit the development of symptoms. An initial set of studies, using human colonic cell lines (CaCo2 and T84) as well as models *in vivo* in mice and chickens (a natural host reservoir for *C. jejuni*), established that acute *C. jejuni* infection increases permeability across the intestinal epithelium, at least in part by disrupting tight junctional claudin proteins. The effects were strain-dependent. These changes promoted the translocation of normal commensal bacteria. The effects can be blocked with administration of Epidermal Growth Factor. We then obtained normal colonic mucosal biopsies from patients undergoing colon cancer screening, through the Intestinal Inflammation Tissue Bank at the University of Calgary. Biopsies were exposed *in culture* to *C. jejuni*, and innate signaling events were assessed. Real-time PCR of gene expression, and the resulting inflammatory response following *in vitro* *C. jejuni* exposure, were analysed to screen for genes involved in *C. jejuni* recognition. Upregulated expression and secretion of inflammatory cytokines IL-1 $\beta$  and GM-CSF was consistent, whereas the common inflammatory markers IL-6 and IL-8 showed greater variability between patients and cell lines. In infected biopsies, *C. jejuni* exposure was associated with the upregulation of recognition receptors TLR2 and TLR9, and NF- $\kappa$ B adaptor proteins TICAM2, TIRAP and TRAF6, but notably not MyD88. These immune pathways were subsequently analysed in more detail using our *in vitro* human cell line models. The Caco-2 cell model confirmed *C. jejuni* stimulation of TLR2 and TLR9 expression

and an inflammatory response independent of MyD88. Activation of the TLR9 pathway was then investigated using polarized T84 cells: *C. jejuni* infection significantly decreased the elevated trans-epithelial electrical resistance induced by apical TLR9 agonists, possibly through disruption of epithelial barrier polarity. These models, used in combination, offer a powerful avenue for future studies of the effects of acute *C. jejuni* enteritis in patients with IBD. Future experiments will use intestinal biopsies of IBD patients in an attempt to detect how abnormalities in these microbial detection systems may lead to the production of inflammatory symptoms.

Prompted by initial experiments in which we found that the effects of *C. jejuni* on paracellular permeability may be strain-dependent, we sought to characterize novel mechanisms whereby *Campylobacter* may induce translocation of non-invasive intestinal bacteria, which in turn may be responsible for an abnormal inflammatory response to commensal bacteria in patients with IBD. Using models of human colonic cell lines as well as mice infected in vivo, the findings indicate that *C. jejuni* may cause internalization of bystander *E. coli* via a transcellular pathway, which may occur independently of any *C. jejuni*-induced increase in intestinal paracellular permeability. The results demonstrated that this may occur via a novel lipid raft-mediated transcellular process. Invasion-defective *C. jejuni* mutants and *Campylobacter*-conditioned cell culture medium also induced *E. coli* translocation, indicating that *C. jejuni* does not directly 'shuttle' bacteria into enterocytes. Therefore, *Campylobacter*, regardless of its own invasiveness, or of its effects on paracellular permeability, may promote the translocation of non-invasive bacteria across the intestinal epithelium.

Overall, the findings characterize new mechanisms whereby *C. jejuni*-induced disruptions of intestinal epithelial function may contribute to symptoms in patients with IBD. An additional parallel study performed in collaboration with Dr. P. Beck found that function of the endothelium was also altered in patients with IBD. IBD patients demonstrated microvascular endothelial dysfunction with lower pulse arterial tonometry indices and shear stress reactive hyperemia compared with controls. Indeed, alteration in vascular flow has also been suggested as a possible mechanism of inflammation in inflammatory bowel disease. Whether endothelial dysfunction may compound the detrimental effects of epithelial abnormalities to cause inflammation in IBD has yet to be assessed.

Summary: The studies show that TLR2 and MyD88-independent immune signaling pathways may be activated by *C. jejuni*, and that *C. jejuni* may stimulate pro-inflammatory responses by changing TLR9 signaling polarity. The data provide initial evidence that human colonic biopsies can be used as an appropriate model for studying the responsiveness of the intestine to *C. jejuni* in healthy hosts, and compare this to what occurs in the intestine of patients with IBD. Results from such investigations may point to novel therapeutic targets, as our findings also indicate that acute *C. jejuni* enteritis promotes the translocation of normal, commensal, bacteria, via mechanisms that could cause post-infectious flare-ups.

## 2. Lay title and summary

Title: The role of acute intestinal infection in the production of symptoms during IBD.

### Summary:

Research findings indicate that intestinal microbes and their interaction with the host contribute to disease in Crohn's disease and ulcerative colitis. *Campylobacter jejuni*, the most common cause of acute intestinal infection, may initiate and/or exacerbate IBD via mechanisms that remain unclear. ***In an attempt to uncover new roads towards therapy, this project aims at understanding 1) how C. jejuni-induced intestinal disruptions may prime the intestine for subsequent inflammation in susceptible hosts, and 2) how Epidermal Growth Factor (EGF) may help protect the intestine upon such interactions.***

Patients with IBD may exhibit abnormal inflammatory responses to their normal intestinal microflora, via mechanisms that remain obscure. Factors that induce translocation of commensal bacteria across the intestine may contribute to the production of symptoms in IBD. Using animal models, human intestinal cell model systems, as well as intestinal biopsy tissues from human patients, our findings to date indicate that:

- 1) *Campylobacter jejuni* strains that can invade the intestinal tissues (but not non-invasive strains) can kill intestinal cells.
- 2) *C. jejuni* may break the intestinal barrier and provoke the penetration of commensal, otherwise non-invasive, intestinal bacteria.
- 3) *C. jejuni* activates classical pro-inflammatory receptors in human intestinal tissues, and changes their polar localization, which in turn will cause abnormal inflammatory reactions to microbes.

4) Epidermal Growth Factor, a natural ingredient of milk, can block the *C. jejuni*-induced injuries, in live animal models as well as in human intestinal cells.

To unravel the mechanisms and consequences of *C. jejuni*-induced host epithelial disruptions will help understand processes that are responsible for intestinal inflammation in IBD. Findings from this project offer novel and exciting explanations of how acute intestinal infections may cause symptoms in patients with IBD. In turn, the discovery of biological agents capable of inhibiting these events may offer insights into new therapeutic strategies.

### 3. List of publications from this grant, and facilitated by CCFC (2008-2009 only)

- Names of A. Buret trainees working on this CCFC grant are underlined -

- **Articles**

1. KALISCHUK, L.D., BURET, A.G. A role for *Campylobacter-jejuni* induced enteritis in inflammatory bowel disease? Am. J. Physiol. (invited review, submitted)
2. KALISCHUK, L.D., INGLIS, G.D., BURET.A.G. (2009) Plasma membrane cholesterol modulates *Campylobacter jejuni* induced translocation of non-invasive *Escherichia coli*. Gut Pathog. 1;2:1-10.
3. ROIFMAN, I., SUN, Y.C., FEDWICK, J.P., PANACCIONE, R., BURET, A.G., LUI, H., ROSTOM, A., ANDERSON, T.J., BECK, P.L. (2009) Patients with inflammatory bowel disease have evidence of systemic endothelial dysfunction. Clin. Gastroenterol. Hepatol. 7(2): 175-182.
4. LAMB-ROSTESKI, J.M., KALISCHUK, L.D., INGLIS, G.D., BURET, A.G. (2008) Epidermal growth factor inhibits *Campylobacter jejuni*-induced claudin-4 disruption, loss of epithelial barrier function, and *Escherichia coli* translocation. Infect. Immun. 76;8:3390-3398
5. O'HARA, J., BURET, A.G. (2008) Mechanisms of intestinal tight junctional disruption during infection. Front. Biosci. (1;13):7008-7021

- **Abstracts**

1. Kalischuk LD, Inglis GD, and Buret AG. Plasma membrane cholesterol modulates *Campylobacter* induced-translocation of non-invasive intestinal bacteria. FASEB J. 2008;23.
2. Kalischuk LD, Inglis GD, and Buret AG. *Campylobacter jejuni* Induces Translocation of

Non-invasive Intestinal Bacteria in vivo and in vitro. Canadian J. Gastroenterol. 2008;22.  
*Trainee award for CDDW 2008.*

3. O'hara JR, Feener TD, Kalischuk LD, and Buret AG. Increased severity of DSS colitis in mice following infection with *Campylobacter jejuni*. Canadian J. Gastroenterol. 2008;22.

4. L Friis, J O'Hara, C Szymanski, P Beck, A Buret. Infection of human colonic biopsies to identify *C. jejuni* recognition receptors. 2009 (submitted)

- ***Oral presentations by A. Buret***

1. June 2009: Speaker, National University of Costa Rica, Faculty of Medicine  
Heredia, Costa Rica

*“Enteric infections and IBD: From bench to bedside”*

2. November 2008: Speaker, Faculty of medicine and applied Sciences,  
Griffiths University, Brisbane Australia

*“Campylobacter enteritis: significance for IBD and novel treatment opportunities”*

3. June 2008: AHFMR visiting lecturer, University of Alberta, Edmonton, Canada

*“Pathophysiology of C. jejuni enteritis: From research to new therapy.”*

#### **4. Supplementary funding**

The present CCFC grant investigates mechanisms whereby acute *C. jejuni* enteritis may contribute to the production of symptoms in patients with IBD. Some of the findings to date indicate that such infections may promote the translocation of normal, non-invasive, commensal bacteria, raising the question of how normal intestinal bacterial biofilms may be modulated in patients with IBD. To tackle these new questions, a new, distinct, collaborative grant with Dr. H. Ceri and Dr. D. McKay (University of Calgary) was awarded as part of the CCFC “Innovations in IBD” competition. The grant was entitled *“The role of biofilms in IBD: Interactions between normal flora, probiotics, and pathogens at intestinal epithelial surfaces as adherent populations”* (\$50,000.00 for 1 year). To date, this one-year research grant has helped train a new graduate student (K. Sproule-Willoughby), and has already yielded very promising results, published in the form of 4 abstracts in 2009, one of which was selected for media presentation at the annual ASM meeting:

1. Sproule-Willoughby, K. M., K. P. Rioux, D. M. McKay, A. G. Buret and H. Ceri. Biofilm formation by gastrointestinal microflora. 2009. Proceedings of the 109<sup>th</sup> Annual General Meeting of the American Society for Microbiology, pp. 101.  
(Abstract was selected by the ASM for media attention)

2. Sproule-Willoughby, K. M., K. P. Rioux, D. M. McKay, A. G. Buret and H. Ceri. 2009. Biofilm formation in the gastrointestinal tract. *Can. J. Gastroenterol.* 23:137A.

3. Sproule-Willoughby, K. M., K. P. Rioux, D. M. McKay, A. G. Buret and H. Ceri. Biofilm formation in the gastrointestinal tract. 2009. Proceedings of the First Annual University of Calgary Grad Conference.

4. Sproule-Willoughby, K. M., K. P. Rioux, D. M. McKay, A. G. Buret and H. Ceri. Biofilm formation in the gastrointestinal tract. 2009. Proceedings of the 6<sup>th</sup> Biannual Banff Inflammation Workshop, pp. 17.

5. **Personnel working on this CCFC grant**

As the trainees hired early on this grant obtained personal awards, additional people could be employed on this project, which enabled the completion of significantly more studies than originally planned. Unused salary could be used for the additional materials required for this larger group of trainees, to the better benefit of this grant.

- *Post-doctoral*

Dr. Jennifer O'Hara

Dr. Lorna Friis

Dr. Jason Fedwick

- *Ph.D student*

Lisa Kalischuk

- *M.Sc. student*

Jennifer Lamb-Rosteski

- *Undergraduate project student*

Heather Sayer

- *Research assistant*

Troy Feener