

## CCFC Grant-in-Aid Progress Report -Year 1 (2008-2009)

**Endocrinological regulation of gut inflammation by serotonin (5-hydroxytryptamine; 5-HT)**

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**Technical Update**

Mucosal changes in inflammatory bowel disease (IBD) are characterized by ulcerative lesions accompanied by a prominent infiltrate of activated cells from both the innate and adaptive immune systems. In addition to immune cells, inflammation in the gut is associated with an alteration in serotonin (5-hydroxytryptamine; 5-HT) producing enterochromaffin (EC) cells. EC cells are the most well characterized subset of gastrointestinal (GI) endocrine cells, which are dispersed throughout the GI mucosa and are the main source of 5-HT in the gut. Changes in intestinal EC cell numbers and 5-HT are observed in patients with IBD and also in experimental colitis. Although the change in EC cells or in 5-HT amount in IBD has been shown to be involved in regulating gut physiology (motility and secretion), it is not clear whether the change plays any role in immune activation and in regulation of gut inflammation. Due to the strategic location of EC cells in gut mucosa, it is very likely that 5-HT plays an important role in immune activation in relation to gut pathology and pathophysiology in various GI disorders including IBD.

The present grant is addressing the role of 5-HT in immune activation and regulation of gut inflammation. Our working hypothesis is that 5-HT plays important role in regulation of gut inflammation by activating and promoting survival of immune cells. We are addressing this hypothesis by breaking the tasks into three aims: i) Determination of the role of EC cells/5-HT in the activation of innate immune cells to promote gut inflammation ii) Elucidation of the ability of 5-HT-conditioned dendritic cells in activating adaptive immune cells in relation to the generation of gut inflammation and iii) Elucidation of the role of EC cells/5HT in apoptosis of immune cells in the context of gut inflammation.

In the period July 2008 to June 2009, we made excellent progress in our studies on elucidating the role of 5-HT in regulation of gut inflammation. We have established the necessary techniques, have done extensive studies on the role of 5-HT in the activation of immune cells and inflammation in gut (Aim 1) and have obtained very interesting and important data from these studies. Utilizing two different models of colitis (DSS and DNBS) we observed delayed onset and decreased severity of clinical disease together with significantly lower macroscopic and histological damage scores in tryptophan hydroxylase1-deficient (TPH1<sup>-/-</sup>) mice which lack 5-HT in gut as compared to wild-type mice, and in mice treated with 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) after induction of colitis. This was associated with down-regulation of macrophage infiltration and production of pro-inflammatory cytokines. 5-HT stimulated production of pro-inflammatory cytokines from macrophages harvested from the peritoneal cavity of wild-type mice and this was inhibited by the addition of NFκB inhibitor suggesting a critical role of NFκB signalling in 5-HT mediated activation of immune cells. Restoration of 5-HT amount in TPH1<sup>-/-</sup> mice by treatment with 5-HT precursor 5-HTP enhanced

the severity of DSS- induced colitis. These results demonstrate a key role of 5-HT in the pathogenesis of inflammation in experimental colitis and endow new insights into the mechanisms of gut inflammation, which may ultimately lead to improved therapeutic strategies to combat inflammatory disorders. Recently this work has been accepted for publication in *GASTROENTEROLOGY*. We have also made significant progress on our studies on the ability of 5-HT to condition dendritic cells in activating adaptive immune cells in relation to the generation of gut inflammation (Aim 2). The work related to these studies of Aim 2 is presented in the recently concluded Digestive Diseases Week at Chicago. We are beginning to study the role of EC cells/5HT in apoptosis of immune cells in the context of gut inflammation (Aim 3).

### **Lay Summary**

Mucosal inflammation in conditions ranging from infective acute enteritis or colitis to IBD is accompanied by alteration in EC cells numbers and 5-HT content in gut. This altered 5-HT response plays an important role in inflammation-induced gut physiological changes like motility and fluid and mucin secretion. Previous studies from my laboratory on immune-mediated alteration of EC cell function and 5-HT production in enteric infection-induced inflammation demonstrated an important role of CD4<sup>+</sup> T cells-derived immune mediators in the up-regulation of EC cells numbers and 5-HT production in gut. Previously by using the DNBS model of experimental colitis, we have shown an increase in EC cells numbers in mucosal inflammation in colon and this increase in EC cells is attenuated in monocyte chemoattractant protein-1 (MCP-1) deficient mice. Consistent with the attenuation in EC cells numbers we observed an amelioration of DNBS-colitis in MCP-1 deficient mice. Although these observations clearly show changes in EC cells and 5-HT during mucosal inflammation, it is not clear whether the change in 5-HT signalling plays any role in regulating gut inflammation. It is very likely that the activated mucosal immune system in enteric inflammation influences EC cell proliferation/differentiation and 5-HT production, which subsequently plays an important role in regulation of gut inflammation. 5-HT can take part in generation of inflammation in gut by influencing the activation of immune cells, increasing production of inflammatory mediators, promoting survival of immune cells and up-regulating the interactions between innate and adaptive immune responses. The studies we are doing with the support from CCFC are aimed to examine the endocrinological regulation of gut inflammation by 5-HT. We are addressing several key unresolved issues, and those are, the elucidation of the role of 5-HT in the activation of immune cells and promotion of gut inflammation, the ability of 5-HT-conditioned innate immune cells in activating adaptive immune cells in relation to gut inflammation, and the role of 5HT in apoptosis of immune cells in the context of gut inflammation.

The studies we have done so far provided us novel information on the role of 5-HT in the regulation of inflammation in gut. We have observed an important role of 5-HT in activating the immune cells (macrophages and dendritic cells) and in generation of gut inflammation. It will be important in the coming year to see if 5-HT has any role in apoptosis of immune cells in relation to gut inflammation. Definitely these studies are important in understanding the role of 5-HT in intestinal pathology and pathophysiology may ultimately lead to improved therapeutic strategies in intestinal inflammatory disorders.

**List of peer reviewed papers and abstracts related to the project or other work facilitated by CCFC funding:**

***Peer-Reviewed Journal Articles***

1. J. E. Ghia, Nan Li, H. Wang, M. Collins, Y. Deng, R. T. El-Sharkawy, F. Cote, J. Mallet & W. I. Khan (2009) Serotonin plays a key role in pathogenesis in experimental colitis. *Gastroenterology* (in press).
2. T. Mizutani, H. Akiho, W. I. Khan, S. Somada, H. Murao, H. Ogino, K. Kanayama and Y. Sumida. (2009). Roles of IL-4, IL-13 and 5-HT in gut motor dysfunction in a murine model of T cell-induced enteropathy in remission. *Neurogastroenterology and Motility* (in press).
3. Y. Motomura, H. Wang, Y. Deng, R. T. El-Sharkawy, E. F. Verdu & W. I. Khan. (2009). helminth antigen based strategy to ameliorate inflammation in experimental model of colitis. *Clinical & Experimental Immunology* 155:88-95.
4. Y. Motomura, J. Ghia, H. Wang, H. Akiho, R. T. El-Sharkawy, M. Collins, Y. Wan, J. T. McLaughlin & W. I. Khan (2008). Enterochromaffin cell and 5-hydroxytryptamine responses to the same infectious agent differ in Th1 and Th2 dominant environments. *Gut* 57:475-481.
5. H. Wang, J. Steeds, Y. Motomura, Y. Deng, M. Verma-Gandhu, R. El-Sharkawy, J. McLaughlin, R. K. Grencis & W. I. Khan (2007). CD4<sup>+</sup> T cell-mediated immunological control of enterochromaffin cell hyperplasia and 5-hydroxytryptamine production in enteric infection. *Gut*. 56: 949-957.

***Peer Reviewed Journal Abstracts***

1. J. Eric-Ghia, N. Li, H. Wang, F. Côté, T. T. El-Sharkawy, J. Mallet and W. I. Khan. (2009). Role of serotonin in immune activation and inflammation in experimental colitis. *Gastroenterology* 136 (5) : M1624, A397
2. H. Wang, J. Eric-Ghia, A. Hirotada, Y. Motomura, F. Côté, J. Mallet and W. I. Khan. (2009). Role of enterochromaffin cells in regulation of gut inflammation and host defense in enteric infection *Gastroenterology* 136 (5) : S1690, A271
3. H. Wang, M. Bogunovic, J. Eric-Ghia, A. Hirotada, J. McClemens, M. Jordana, L. Mayer and W. I. Khan. (2009). IL-13 plays a critical role in development of enterochromaffin cell hyperplasia and production of serotonin. *Gastroenterology* 136 (5) : S1616, A235
4. J. Eric-Ghia, H. Wang, Y. Deng, N. Li, R. T. El-Sharkawy, F. Côté, J. J. Mallet & W. I. Khan (2008). An essential role of 5-HT in pathogenesis of experimental colitis. *Gastroenterology* 134(4): Suppl. 1 : A218

5. J. Eric-Ghia, H. Wang, N. Haq, Y. Deng, R. El-Sharkawy, A. Velcich & W. I. Khan (2008). Muc2 deficiency interfered in host defense against nematode infection. *Canadian Journal of Gastroenterology* 22: 83A
6. JH. Akiho, H. Murao, H. Ogino, K. Kanayama, Y. Sumida, S. Yoshinaga, S. Itaba, K.Nakamura, R.Takayanagi, W. I. Khan, Y.Tokita, K. Satoh, M. Nishiyama, A.Kaneko & M. Yamamoto (2008). Role of acetylcholine and serotonin receptors in the maintenance of muscle hypercontractility in a murine model of post-inflammatory irritable bowel syndrome. *Gastroenterology* 134(4): Suppl. 1 : A9