学 位 論 文 の 要 旨

論文題目　Regulation of suppressor of cytokine signaling 1 in the intestine by dietary fibers
（食物繊維による腸管サイトカインシグナル抑制因子の調節作用に関する研究）

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1. Introduction and Literature review (Chapter 1-2)

The intestinal epithelium is dynamic and effective in maintaining a distinct physical and biochemical barrier for a constant state of homeostasis. It works under intense conditions and relies on tight cellular and molecular control of mechanisms as means of adaptation to constant antigen pressure. However, continual exposure to pathogens and other luminal noxious molecules lead to loss of barrier function and innate immunity, triggering inflammatory responses. This stimulation of proinflammatory cascade produce different inflammatory cytokines which leads to further inflammatory pathways culminating into mucosal inflammation, probably the basis of inflammatory bowel diseases. Studies have appreciated the critical role of intercellular proteins such as suppressor of cytokine 1 (SOCS1) in suppressing cytokine signaling of proinflammatory cytokines through the Janus activated kinase (JAK)/ signal transducers and activators of transcription (STAT) pathways. SOCS1 proteins negatively regulate the JAK/STAT signaling pathway by directly inhibiting catalytic activity of JAK tyrosine kinase. Deficiency in SOCS1 leads to chronic inflammation induced by proinflammatory cytokines such as interferon g, tumor necrosis factor-a, interlukin-1b, -6 and others.

Accumulating evidence show various beneficial health effects of supplemental fermentable dietary fibers, such as guar gum (GG) and its hydrolysate partially hydrolyzed GG (PHGG). Previously, our research group established that GG fiber increased short chain acids (SCFAs) production which may have contributed to suppression of inflammatory immune responses and reduced barrier defects in the colon of colitic mice. It was also demonstrated that intact GG directly upregulated SOCS1 expression, regulating inflammatory responses in the small intestine through activation of toll-like receptor (TLR)-2 and dectin-1 signaling pathways. However, the regulation of the intestinal SOCS1expression was not validated *in vivo* so far. In addition, the effects of GG and the underlying mechanisms could differ between the small intestine and colon.

The purpose of this study was to investigate the role of dietary fibers particularly GG and PHGG on regulation of intestinal SOCS1 and examine the microbial activity *in vivo*. *In vitro* study used human intestinal Caco-2 to verify the impact of SCFAs on SOCS1 expression.

2. Guar gum fiber uses different mechanisms to upregulate SOCS1 in the small intestine and colon in mice.

Chapter 3: The study aimed at validating upregulation of SOCS1 expression in the small intestine and also investigate the effect of the fiber on SOCS1 expression in the colon. Different

concentrations (5 and 10% GG fiber) were used for this study. The results showed that only the higher concentration upregulated SOCS1 in the small intestine suggesting that the epithelial cells directly recognized the specific structure of intact GG, through activation of TLR-2 and dectin-1 pathways. However, the luminal concentration of the intact GG may matter, hence no effect on SOCS1 expression was evident for the lower concentration. In the colon, both concentrations upregulated SOCS1 and correlated positively with increased SCFAs production, suggesting that supplemental GG can induce SOCS1 expression more effectively in the colon than the small intestine even at low dietary levels. Hence, it was concluded that GG fiber induce SOCS1 in the small intestine in its intact form while in the colon, microbial metabolites might be responsible for the induction of SOCS1 expression.

3. Fermentable fibers increase SCFAs and upregulate SOCS1 expression in mice colon.
Chapter 4: The objective of the study in this chapter was to compare the influence of GG and PHGG on intestinal SOCS1 expression and SCFAs production. Technological treatment permits modification of the physiochemical properties to optimize the functional and physiological properties of fibers. PHGG is a hydrolysate of GG with same chemical structure but different molecular size, hence lower viscosity. At the same concentration, only GG fiber induced SOCS1 expression in the small intestine. However, in the colon, both fibers induced SOCS1 expression in a similar manner which was equally correlated to SCFAs production. It was concluded that specific structure of the intact GG fiber is responsible for induction of the protein expression in the small intestine but SCFAs may be responsible for PHGG/GG-mediated SOS1 expression in the colon since both fibers undergo fermentation by gut microbiota.

4. Antibiotic administration suppresses guar gum fiber-mediated SOCS1 expression in the colon
Chapter 5: This chapter examined the role of microbial activity with associated SCFAs production in SOCS1 expression. Antibiotic administration negatively influenced the colonic SOCS1 expression as well as SCFAs production. On the other hand, upregulation of SOCS1 was observed in mice without antibiotic administration in a similar manner as in previous chapters. A correlation was also observed on SOCS1 expression and SCFAs production. It was concluded that microbial fermentation is involved in increased colonic SOCS1 expression by GG as antibiotic administration reduced microbial activity, consequently affecting SOCS1 expression in the colon.

5. Butyrate influences SOCS1 upregulation in intestinal Caco-2 cells.
Chapter 6. This chapter assessed the role of SCFAs in SOCS1 expression using human intestinal Caco-2 cells. Short chain fatty acids (SCFAs) are main end products of intestinal microbial fermentation of nondigestible polysaccharides. They are known to be influential in various physiological effects including intestinal homeostatic regulation through modification of various cellular processes. The results of this study showed that among the tested SCFAs, acetate, propionate, butyrate, valerate and their isoforms, only butyrate showed potential in inducing SOCS1 expression in Caco-2 cells. It was concluded that SOCS1 expression in *in vitro* studies is sensitive to butyrate only unlike other SCFAs.

6. Overall conclusion (in Chapter 7)

The results of the entire study revealed that GG fiber uses different mechanisms in regulating intestinal SOCS1. In the small intestine the fiber appears to upregulate SOCS1 in

its intact form through TLR2 and Dectin-1 pathways as reported by previous researchers in our laboratory. In the colon, GG-mediated SOCS1 upregulation was influenced by microbial activity since antibiotic administration reduced SCFAs production and suppressed SOCS1 expression. However, GG hydrolysate could not upregulate SOCS1 in the small intestine, but the colon, suggesting that the specific structure of intact GG may play a role in SOCS1 expression. For the *in vitro* study, the results suggest that butyrate is the possible candidate responsible for upregulation of SOCS1 in Caco-2 cell.

Since SOCS1 targets proinflammatory cytokines that lead to inflammatory diseases, dietary interventions that increase intracellular levels of SOCS1 protein may be an alternative approach in the fight against inflammatory diseases.