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한약재인 황금의 궤양성 실험동물에 대한 장간막 임파절 임파구의 면역글로블린 수준, T세포 집단, 사이토카인 생성의 조절작용

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Scutellaria baicalensis Modulates Cytokine Production, T Cell Population and Immunoglobulin Level by Mesenteric Lymph Node Lymphocytes in Experimental Mice with Colitis

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ABSTRACT : We previously examined extracts, isolated from *Scutellaria baicalensis* (SB), chemical mediators, and IgE by mesenteric lymph node (MLN) lymphocytes in rats. The present study was to evaluate the effects of extracts of SB on the MLN lymphocytes function of mice given orally by 20 ㎎/㎏ for 2 weeks with dextran sulfate sodium (DS)-induced colitis. Results show that IgE levels in MLN lymphocytes was low, while IgA was high, in mice given SB compared to that fed water. Concentrations of inteferon-γ and interleukin (IL)-2 of T cells by concanavalin A treatment was significantly higher in the SB fed group than the normal group. Activation-induced IL-4 and IL-10 secretion was lower in SB fed mice compared control mice after DS-induced colitis. These results suggested that SB suppresses the inflammation in DS-induced colitis through the modulation of Th1/Th2 balance to down-regulate Th_2 response in MLN lymphocytes.

Key Words **:** *Scutellaria baicalensis*, IgE and IgA, Inflammatory Bowel Disease, Mesenteric Lymph Node

INTRODUCTION

Chinese herbal medicine has recently been of increased interest for the treatment of these disorders. We previously examined extracts, isolated from *Scutellaria baicalensis* (SB), chemical mediators, and IgE by mesenteric lymph node (MLN) lymphocytes in rats. In addition, SB ethanol extracts have very potent antioxidative action in vivo as well as in vitro (Lim *et al.,* 1998 and 1999; Choi *et al.,* 2000), and much more potent than β-carotene (Kim *et al.,* 1996). We also reported SB extracted from has immunoregulatory action in vivo as well as in vitro (Lim *et al.*, 2007). However, the study about the inflammatory bowel disease (IBD) of SB has not been totally appreciated. We therefore examined the effect of SB administration on IBD by using mesenteric lymph node (MLN) lymphocytes isolated from Balb/c mice.

IBD including Crohn's disease (CD) and ulcerative colitis (UC) is chronic, relapsing, and remitting condition of unknown origin that exhibits various features of immunological inflammation (Blumberg *et al.*, 1999). Studies in humans have implicated impaired mucosal barrier function, pronounced innate immunity, production and proinflammatory and immunoregulatory cytokines, and the activation of $CD4^+$ T cells in the pathogenesis of IBD (Fiocchi 1998., Lim *et al.*, 2002). There is clear evidence supporting a role for cytokine in the initiation and perpetuation of IBD (Fiocchi 1992). Recent studies have shown that the production of the proinflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8 at sites of inflammation is markedly enhanced in both patients with UC and CD (Sartor *et al.,*

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1994). Therapeutic trials of cytokine manipulation in such patients further support the idea that cytokines are important in the pathophysiology of these diseases (Schreiber *et al.*, 1995).

Many studies have shown the medicinal crops are an important role for the intestinal structure and function (Kiyohara *et al.*, 2002). On the other hand, very few studies have focused on the effects of SB on chemotherapeutic-induced colitis. The purpose of this current study was to evaluate the intestinal immunoregulatory effects of SB on DS induced colitis in mice. Oral administration of DS in mice induces colitis resembling human UC. This model corresponds well to the clinical signs of IBD in human and can serve as a reliable model for studies of this disease (Blumberg *et al.*, 1999). We focused this study on the response of MLN lymphocytes in mice after DS-induced colitis, and investigated the effect of SB on Ig, T cell and cytokine production.

MATERIAL AND METHODS

1. Materials

Concanavalin A and dextran sulfate sodium (DS) were purchased from Sigma (St. Louis., Mo, USA). Hemoglobin reagent (AM 503-K) was purchased from Asan Pharmaceutical Co. (Ltd. Seoul, Korea). This compound was dissolved in phosphate buffered water (PBS, pH 7.4) or water and used for cell culture experiments. Monoclonal antibodies and cytokines were purchased from ID Labs Inc. (Ontario, Canada). IgA related antibodies purchased from Zymed Laboratories Inc. (Sanfrancisco, CA, USA). IgE related antibodies purchased from Biosource International (Comarillo, CA, USA). For the enzyme-linked immunosorbent assay (ELISA) of rat IgE and IgA, 0.05% Tween 20 in PBS (TPBS) for rinsing and Block Ace (Dainihon Pharmaceutical Co., Osaka, Japan) were used for blocking and dilution of antibodies as described previously (Lim *et al.*, 2000, 2007b). All other reagent grade chemicals were purchased from Sigma (St. Louis., MO, USA).

2. Preparation of *Scutellaria baicalensis* (SB) extracts

Scutellaria baicalensis was ground to a fine powder with a grinder. Powder (3 kg) was extracted with 70% ethanol for 3 hr at room temperature. The extract was filtered, and the filtrate was concentrated under reduced pressure.

3. Induction of colitis

Colitis was induced by means of drinking water supplemented with 5% DS. This model has been described in detail previously (Murthy *et al.*, 1993). Normal mice were fed in a similar manner with drinking water containing no DS.

4. Study design and diets

Four-week old female Balb/c mice were obtained from the Samtako Bio Korea (Osan, Korea). All animal-care techniques were performed within the guidelines approved by the Institutional Animal Care and Use Committee. The animals were maintained on a 12-h dark-light cycle and allowed free access to nonpurified pellet diet and tap water under conditions of controlled temperatures (25 ± 2℃). They were adapted for 7d prior to initiation of the experimental protocol. The mice allowed AIN-93G diet (Reeves *et al.* 1993) and drinking water ad libitum. All mice were divided into five groups of 7 mice. The mice were divided into four groups as follows: (1) Normal group- This group was fed AIN-93G diet and drinking water without DS for 5 days. After 5 days, it was orally treated with water only for 2 week. (2) DS group -Acute colitis was induced by feeding mice for 5 days with DS. After 5 days of DS, when mice returned to drinking plain water, the mice were treated with water only for 2 week. (3) SB group-This group was fed AIN-93G diet and drinking water without DS for 5 days. SB was given orally at 20 ㎎/㎏ for 2 weeks. Group (4) DS + SB group-Acute colitis was induced by feeding mice for 5 days with 5% DS. After 5 days of DS, when mice returned to drinking plain water, the mice were given orally SB at 20 ㎎/㎏ for 2 week. After body weight and blood hemoglobin content were measured, and then the medical longitudinal length was then measured.

5. Preparation of mesenteric lymph node (MLN)

MLN was excised from Balb/c mice and lymphocytes were squeezed out into the RPMI 1640 medium (Invitrogen Corporation, Grand Island NY, USA). After incubating the cells at 37℃ for 30 min to remove fibroblasts, 5 ㎖ of the cell suspension was layered on 4 ㎖ of Lympholyte-mice (Cedarlane, Hornby, Canada) and centrifuged at 1,500 *xg* for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed three times with the

RPMI 1640 medium. The lymphocytes were cultured in a 10% FBS (Invitrogen Corporation, Grand Island NY, USA)/ RPMI 1640 medium, and IgE and IgA content of the culture supernatant were measured by ELISA (Engvall and Perlman 1971). Cell viability was measured by trypan blue staining. Cell viability by this preparation was more than 95% of the total cells.

6. Enzyme-linked immunosorbent assay of mice antibodies

Measurements of IgE and IgA were executed using sandwich ELISA methods, as reported previously by Lim *et al.* (2000).

7. Isolation of mesenteric lymph node (MLN) T-lymphocytes subsets

To the MLN lymphocytes suspended at 1×10^6 cells/ 100 ㎖, 10% FBS/PBS was added 5 ㎖ of either CD4-FITC or CD8-PE monoclonal antibodies (Santa Cruz, CA. USA), and incubated at 4℃ for 30 min. The lymphocytes were rinsed three times with PBS containing 10% FBS and centrifuged at 1,200 rpm for 5 min. The stained lymphocytes were fixed by 2% paraformaldehyde and were counted by Epics Altra™ flow cytometry (Beckman Coulter, USA). Each analysis, including those of negative control samples, was based on at least 10^4 events after dead cells and gating on the basis of forward angle light scatter eliminated residual erythrocytes (Lim *et al.*, 2000).

8. Measurement of cytokines

Supernatants from 48h ConA-activated MLN lymphocytes culture were obtained. Cytokines (IFN-γ, IL-2, IL-4 and IL-10) were measured by ELISA using cytokine-specific capture and detection monoclonal antibodies as previously described (Fernandes 1999).

9. Statistical analysis.

The data are presented as mean ± SD. Differences between the means of the individual groups were assessed by one-way ANOVA with Duncan's multiple range tests (1955).

RESULTS

1. Growth variables

In the case of the food intake and body weight, the each mouse in the dextran sodium sulfate (DS) and DS + SB was significantly lower than that of mice fed water or SB (Table 1A). As shown in Table 1B, the colon length of the DS-treated mice was significantly shorter than those of normal mice or SB. The severity of ulcerative colitis-like lesions was most marked in the large intestine on the 5th day. The administration of SB significantly relieved all these symptoms. The hemoglobin content of those treated with SB was significantly higher than that of the DS group.

Table 1A. Food intake and body weight of mice fed on the *Scutellaria baicalensis* extracts for 2 weeks.

Groups	Initial body weight (g)	Final weight (g)	Food intake
Normal	18.8 ± 1.1^a	23.0 ± 1.0^a	$13.1 \pm 0.3^{a*}$
DS	18.3 ± 0.6^a	17.3 ± 0.3^b	11.2 ± 0.4^b
SB	19.1 ± 0.6^a	21.6 ± 0.6^a	13.0 ± 0.3^a
DS + SB	18.1 ± 0.5^a	17.5 ± 0.3^b	11.4 ± 0.7^b

Values represent the means ± SD of seven mice. * Values with different superscripts are significantly different ($p < 0.05$).

Table 1B. Effect of *Scutellaria baicalensis* on colon length and blood hemoglobin.

Groups	Colon length (㎝)	Blood hemoglobin (g/dℓ)
Normal	11.2 ± 0.5^a	12.0 ± 0.1^a
DS	6.7 ± 0.4^b	8.4 ± 0.4^b
SB	10.7 ± 0.2^{bc}	11.1 ± 0.2^a
DS + SB	7.8 ± 0.5^c	10.8 ± 0.5^a

Values represent the means ± SD of seven mice. * Values with different superscripts are significantly different ($p < 0.05$).

2. Mesenteric lymph node (MLN) Ig concentration

IgA concentration of MLN lymphocytes in the absence of ConA was higher in mice fed SB than in those fed water (Fig. 1). It was significantly lower in the DS group than in normal, SB and DS + SB group. ConA treatment increased the concentration of IgA in cell from water and SB fed groups, and the value was markedly high in the former. IgA concentration in MLN lymphocytes from DS + SB group was higher than in cells from the DS group. On the contrary, IgE concentration in MLN lymphocytes from mice DS was higher than in those fed DS + SB when ConA was absent. When the cells were cultured with ConA, similar response patterns were observed.

3. T cell population of MLN lymphocytes

There was a significant increase of the relative population of $CD4^+$ T cell in DS-induced colitis group compare to

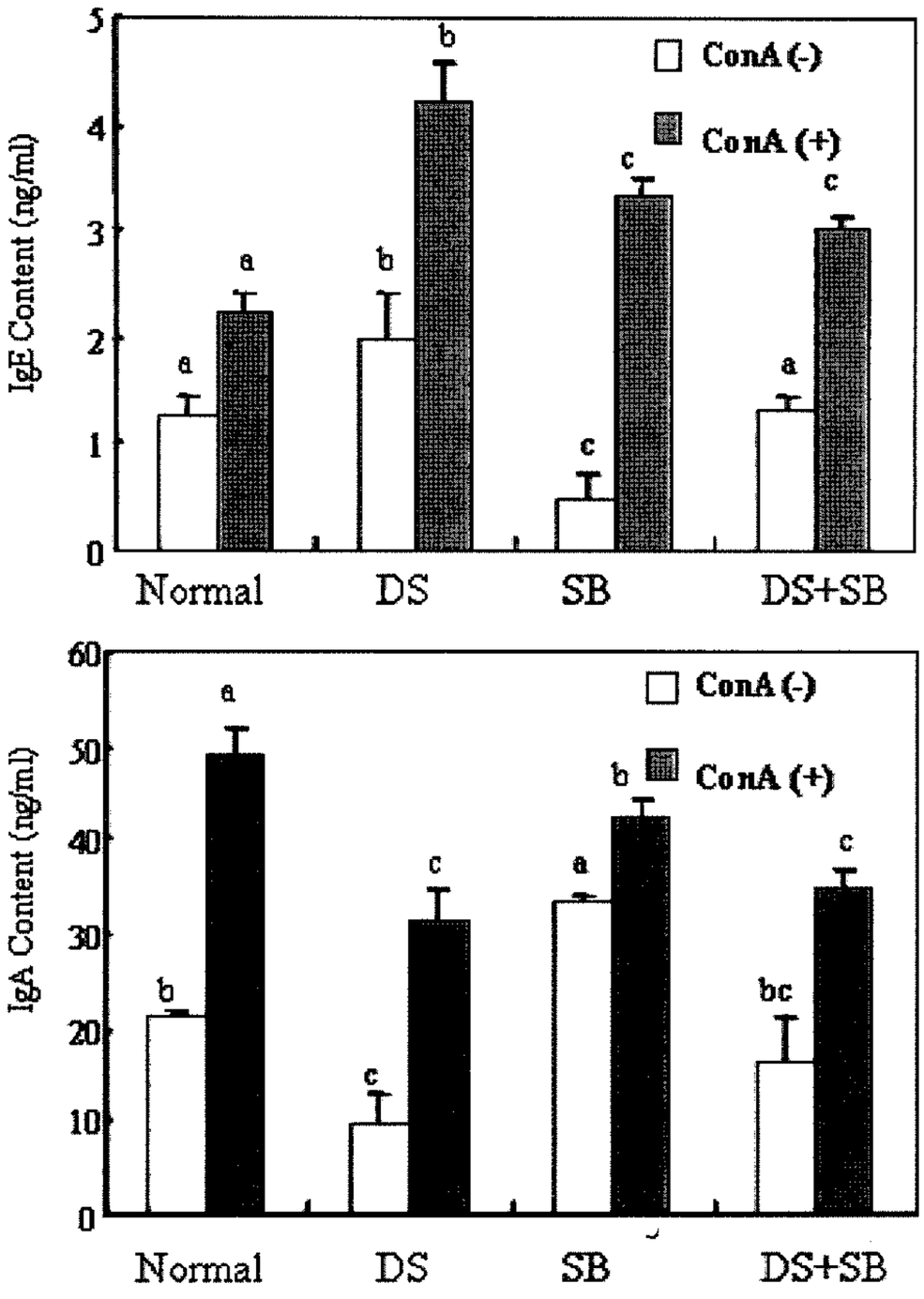

Fig. 1. IgA and IgE Production by Mesenteric Lymph Node Lymphocytes Isolated from Mice Fed on SB extracts. MLN lymphocytes isolated from mice fed SB extracts (2 x 10^6 cells/㎖) were cultured for 48 hr in the absence (white bars) and presence (screened bars) of 25 ㎎/L of concanavalin A (ConA). Ig contents of culture supernatants were measured by ELISA. The results are the means ± SD (n = 7). $^{a-d}$ Values not sharing a common letter are significantly different at $p < 0.05$.

Table 2. Mesenteric lymph node lymphocytes population in normal and DS-induced colitis in mice treated with *Scutellaria baicalensis* extracts.

(Unit: %)

Groups	$CD4^+$	$CD8^+$
Normal	49.4 ± 1.7^a	11.1 ± 0.4^a
DS	56.0 ± 2.4^b	9.7 ± 0.5^b
SB	52.1 ± 1.2^c	10.7 ± 0.5^a
DS + SB	56.0 ± 1.8^b	11.1 ± 0.4^a

Values represent the means ± SD of seven mice. *Values with different superscripts are significantly different ($p < 0.05$).

those fed water and SB (Table 2). On the other hand, the proportion of $CD8^+$ cells higher in the normal group than the SB group. However, the proportion of $CD8^+$ in normal and DS + SB group remained unchanged.

Table 3. Activation-induced Th1 and Th2 cytokine secretion in MLN lymphocytes in dextran sulfate sodium-induced colitis in mice.

(Unit: pg/㎖)

Groups	Th1		Th2	
	IFN-γ	IL-2	IL-4	IL-10
Normal	666 ± 14^a	86 ± 3^a	53 ± 3^a	57 ± 2^a
DS	568 ± 24^b	146 ± 10^b	113 ± 7^b	76 ± 4^b
SB	811 ± 49^c	174 ± 11^c	57 ± 8^a	57 ± 10^a
DS + SB	896 ± 34^d	197 ± 5^d	48 ± 2^c	48 ± 6^a

Values represent the means ± SD of seven mice. $^{a-d}$ Values with different superscripts are significantly different ($p < 0.05$).

4. Cytokine concentrations in MLN lymphocytes

When lymphocytes were cultivated for 48 h without ConA, the concentrations of IFN-γ and IL-2 were below detection limit (data not shown). In the presence of ConA, IFN-γ and IL-2 concentration were highest in cell from the SB group (Table 3). As these results, SB group in the DS induced colitis showed higher production IFN-γ and IL-2 than normal or DS group. On the other hand, DS induced colitis was accompanied by a disturbance in IL-4 and IL-10. Activation with ConA significantly enhanced IL-4 and IL-10 production in the DS-induced colitis. IL-4 and IL-10 concentration of the SB fed group not different than of mice fed normal group. Interestingly, mice fed SB in the DS-induced colitis group showed lower production of IL-4 than in DS groups. However, the production of IL-10 in SB, DS + SB unchanged.

DISCUSSION

Lymphocytes in the intestinal mucosa first interact with antigens in the organized lymphoid tissues (Peyer's patches and lymphoid follicles in the colon) and further differentiate and mature in the germinal centers of the lymphoid follicles. Thereafter, they rapidly leave the mucosa and migrate through the MLN and the thoracic duct to reach the systemic circulation (James and Kiyono, 1999). Their well-known immune roles are well publicized for their putative efficacy against the incidence of major diseases such as arteriosclerosis, cancer and diabetes (Brandi 1992). We chose to study this component because of our previous finding of their potent immune effect. In the present study, we attempted to document SB on immunological activity in an inflammatory bowel disease animal

model system.

The effect of inflammatory bowel disease on SB in immunological function is not fully understood. As shown in Fig. 1, IgA productivity during 48-hr incubation of MLN lymphocytes was significantly higher in the DS + SB group than in those DS group. Since IgA plays a crucial role in the prevention of allergic reaction through interference with allergen absorption (Metcalfe 1991), this effect seems worthwhile. Increase of IgA concentration in clinical diseases animals might reflect an increase in immunoglobulin-mediated mucosa protection.

On the contrary, IgE productivity in the SB groups was significantly lower than that in the normal group. In the case of DS induced colitis similar response patterns were observed. SB enhanced IgA production and reduced IgE production by the lymphocytes in DS induced colitis. For this reason, SB was expected to alleviate inflammatory reaction in intestinal immune system.

Our data clearly shown that SB mediated IgA and IgE production by the MLN lymphocytes in DS induced colitis (Fig. 1). If so, it may possible that administration SB regulates the immune response induced by helper T cells (Brandi 1992). Chinese medicine characteristically modified the proportion of $CD4^+$ and $CD8^+$ T cells in the MLN lymphocytes (Zang *et al.,* 2004). $CD8^+$ T cells regulate $CD4^+$ T cells development by producing IFN-γ or other regulatory cytokines that suppress the development of Th2 cells and may favor for Th1 cell growth (Cher and Mossman 1987). As shown in Table 2, there was a significant increase of the relative population of $CD4^+$ T cell in DS induced colitis group. DS + SB group was similar in normalizing the $CD4^+$ to $CD8^+$ ratio, indicating the effect to be mediated by administration SB. As a result, the effect of SB is expected to mediated, at least in part, through an influence on the differentiation of T cells to become Th1 cells.

The cytokine profile of UC mucosa shows Th2 features, while CD is believed by some to be a Th1-mediated disease. Th1 cells mediate delayed-types hypersensitivity, allograft rejection, and certain autoimmune disease. Abnormal Th2 response is implicated in atopic disease. The cytokines are important factors involved in inflammation and regulation of the immune response. IFN-γ, IL-2, TNF-α, IL-4, IL-5 and IL-10 are important in the initiation, regulation and perpetuation of inflammation in UC and CD (Obermeier *et al.,* 1999). In the present study, we showed that the effect of fed SB on IFN-γ and IL-2 was strongly, but IL-4 and IL-10 concentrations higher in mice with DS-induced colitis than in normal group (Table 3). We demonstrated that increased Th1 cell's cytokine and decreased Th2 cell's cytokine could be reversed by fed with SB even after the inflammation had been persistent. These data may suggest that treatment of DS-induced colitis with fed SB will strengthen the immune system in colitis by regulating cytokines such IL-4 and IL-10, protecting against the colitis-related damage. Therefore, The inhibitory effect of IL-4, IL-5 and IL-10 of administration in DS-induced colitis could be mediated by the influence of IFN-γ and IL-2 by Th1 cells.

We demonstrate that SB can alleviate the inflammation by abnormal Th2 response in DS induced colitis. In addition, SB enhanced IgA production and reduced IgE production by the MLN lymphocytes in DS induced colitis. Although the exact mechanism by which SB modifies immune indices is not apparent at present, the current observations can open a new aspect of the immunological role in inflammatory bowel disease of fed SB.

Conclusion

Our study was to evaluate the effects on extracts of *Scutellaria baicalensis* (SB) on the MLN lymphocytes function of mice given orally at 20 ㎎/㎏ for 2 weeks with dextran sulfate sodium (DS)-induced colitis. Results show that IgE levels in MLN lymphocytes was low, while IgA was high, in mice given SB compared to that fed water. Concentrations of inteferon-γ and interleukin (IL)-2 by concanavalin A treatment was significantly higher in the SB fed group than the normal group. Activation-induced IL-4 and IL-10 secretion was lower in SB fed mice compared control mice after DS-induced colitis. We suggested that SB suppresses the inflammation in DS-induced colitis through the modulation of Th1/Th2 balance to down-regulate Th2 response in MLN lymphocytes.

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LITERATURE CITED

Blumberg RS, Saubermann LJ, Strober W (1999) Animal models of mucosal inflammation and their relation to human inflammatory bowel disease. Curr Opin Immunol 11:648-656.

Brandi ML (1992) Flavonoids: biochemical effects and therapeutic applications. Bone Mineral 19:S13-S14.

Cher DJ, Mosmann TR (1987) Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated by TH1 clones. J Immunol. 138:3688-3694.

Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK (2000) Application of flow injection-chemiluminescence to the study of radical scavenging activity in plants. Phytotheraphy Res 14:250-253.

Duncan DB (1955) Multiple range and multiple F test. Biometrics 11:1-42.

Engvall E, Perlman P (1971) Enzyme-liked immunosorbent assay (ELISA), quantitative assay of immunoglobulin G. Immunochemistr. 8:871-874.

Fernandes G (1999) Methods for the study of immune cells in aging. In Methods in Aging Res. CRC Press Salem, MA, p. 549-567.

Fiocchi C (1992) Cytokine. In Inflammatory Bowel Disease Mac Dermort R and Stenson W (eds). Elsevier Press New York, p. 137-162.

Fiocchi C (1998) Inflammatory bowel disease: etiology and pathogenesis. Gastroenterology 115:182-205.

James SP, Kiyono H (1999) Gastrointestinal lamina propria T cells. In: Mucosal Immunology Ogra PL, Mestecky J, Lamm ME, Strober W, Bienenstock J, McGhee JR (eds) San Diego: Academic Press, p. 381-396.

Kim SC, Choi OB, Oh JH, Park DK (1996) Effects of ganhuangenin obtained from *Scutellaria Baicalensis Georgi* on the peroxidation of phospholipid. Biennial meeting international society for free radical research. 8, p. 114.

Kimura Y, Okuda H, Tani T, Arichi S (1982) Studies on Scutellaria baicalensis. VI. Effect of flavanone compounds on lipid peroxidation in rat liver. Chem Pharm Bull 30:1792-1795.

Kiyohara H, Matusmoto T, Yamada H (2002) Intestinal immune system modulating polysaccharides in a Japanese herbal (Kampo) medicine. Juzen-Taiho-To Phytomedici, 7:614-624.

Lim BO, Yu BP, Cho SI, Her E, Park DK (1998) The inhibition by quercetin and ganhuangenin on oxidatively modified low density lipoprotein. Phytotheraphy Res 12:340-345.

Lim BO, Yu BP, Kim SC, Park DK (1999) The antioxidative effect of ganhuangenin against lipid peroxidation. Phytotheraphy Res 13:479-483.

Lim BO, Yamada K, Nonaka M, Kuramoto Y, Hung P, Sugano M (1997) Dietary fibers modulate indices of intestinal immune function in rats. J Nutr 127:663-667.

Lim BO, Jolly CA, Zaman K, Fernandes G (2000) Dietary (n-6) and (n-3) fatty acids and energy restriction modulate mesenteric lymph node lymphocyte function in autoimmune-prone (NZBxNZW)F1 mice. J Nutr 130:1657-1664.

Lim BO, Yamada K, Choue RW (2002) The Key role of cytokine in immunopathogenesis of inflammatory bowel disease. Report of Japanese Association for Biological Function Research 6:4-17.

Lim BO, Choi SY, Choi DK, Park PJ, Choi WS, Kim JD, Shin HM (2007a) Inhibitory Effect of *Scutellaria baicalensis* Root Extract on Chemical Mediator Release and Immune Response. Korean J. Medical Crop Sci. 15:132-137.

Choi SY, Lee KC, Jeoung YJ, Lim BO (2007b) In-vitro Anti-inflammatory Activity of *Rubus coreanus Miq.* on Nitric Oxide, Interferon-γ, Cycloxygenase-2, and Tumor Necrosis Factor-α Production in the Macrophage like Cell Line RAW 264.7 Activated by Lipopolysccharide. Korean J. Medical Crop Sci. 15:324-328.

Metcalfe DD (1991) Food allergy. Curr Opin Immunol 3:881-886.

Murthy SNS, Cooper HS, Shim H, Shah RS, Inrahim SA, Sedergran DJ (1993) Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. Dig Dis Sci 38:1722-1734.

Obermeier F, Kojouharoff G, Han W, Scholmerich J, Gross V, Falk, W (1999) Interferon-gamma (IFN-α) and tumor necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulfate sodium (DSS)-induced colitis in mice. Clin Exp Immunol 116:238-245.

Reeves PG, Nielsen FH, Fahey GC (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 123:1939-1951.

Sartor RB (1994) Cytokines in intestinal inflammation: pathophysiological and clinical considerations. Gastroenterology 106:533-539.

Schreiber S, Heing T, Thiele HG, Raedler A (1995) Immunoregulatory role of interleukin 10 in patient with inflammatory bowel disease. Gastroenterology 108:1434-1444.

Zhang CP, Tian ZB, Liu XS, Zhao QX, Wu J, Liang YX (2004) Effect of Zhaoyangwan on chronic hepatitis B and post cirrhosis. World J Gastroenterol 15:295-298.