Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1092–1099

Journal of Nutritional Biochemistry

Pretreatment with alanyl-glutamine suppresses T-helper-cell-associated cytokine expression and reduces inflammatory responses in mice with acute DSS-induced colitis☆

Chia-Chou Chu[a], Yu-Chen Hou[b], Man-Hui Pai[c], Chen-Jui Chao[b], Sung-Ling Yeh[b,*]

[a]Graduate Institute of Pharmacy, Taipei Medical University, Taipei, Taiwan
[b]School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan
[c]Department of Anatomy, Taipei Medical University, Taipei, Taiwan

Received 17 March 2011; received in revised form 4 May 2011; accepted 9 June 2011

Abstract

T-helper (Th) cells play a major role in initiating and shaping the pathologic response in inflammatory bowel disease (IBD). Glutamine (GLN) is a nutrient with immune-modulating effects. This study investigated the effect of GLN on cytokine expressions and inflammatory responses of three subsets of Th cells in dextran sulfate sodium (DSS)-induced IBD. There were one normal control (NC) and two DSS groups. Mice in the DSS groups drank distilled water containing 3% DSS for 5 days, whereas the NC group received distilled water. Mice in the G-DSS group were given intraperitoneal injection of 0.5 g GLN/kg/d for 3 days before receiving DSS water. The other DSS group (C-DSS) received an identical amount of amino acid solution without GLN. After induction of IBD, the mice were allowed to recover for 3 days and then were sacrificed. Blood and colon samples were collected for further analysis. The C-DSS group had higher percentages of blood interleukin (IL)-17A, IL-17F, IL-22, IL-4 and interferon-γ than the NC group. The G-DSS group had lower Th1/Th17/Th2 cytokine expressions, which showed no differences from the NC group. Plasma haptoglobin, colon immunoglobin G and chemokine levels and myeloperoxidase activities were higher in the DSS groups than the NC group. These parameters were significantly lower in the G-DSS than the C-DSS group. These results suggest that pretreatment with GLN suppressed Th-associated cytokine expressions and may consequently reduce inflammatory mediator production and leukocyte infiltration into tissues, thus ameliorating the severity of acute DSS-induced colitis.

Keywords: DSS colitis; Glutamine; T-helper cells; Haptoglobin; Immunoglobin G

1. Introduction

Inflammatory bowel disease (IBD) is a complex multifactorial disease of unknown etiology. It comprises two major forms of chronic inflammatory disorders of the gastrointestinal tract, Crohn's disease and ulcerative colitis (UC), which are characterized by distinct clinical histopathological and endoscopic features. It is widely accepted that the pathogenesis is likely dependent on interactions between luminal immune reactions and environmental factors in genetically susceptible individuals [1]. Studies of experimental models of IBD suggest that this immunopathological process consists of an excessive and dysregulated immune response to components of the bacterial microflora [2]. CD4+ T-cells play a major role in initiating and shaping this pathologic response [3]. T-helper (Th) cells are traditionally divided into two distinct subsets, Th1 and Th2, characterized by distinct cytokine profiles and effector functions. Recently, an additional Th cell subset which preferentially produces interleukin (IL)-17 was found and designated Th17. There is dynamic cross-regulation among the Th1, Th2 and Th17 pathways. The proper balance of this cross-regulation is critical for optimizing defenses to microbes while simultaneously avoiding chronic tissue inflammation in IBD [4].

Glutamine (GLN) is the most abundant free amino acid in the plasma and tissue pool. It is a critical substrate for enterocytes and rapidly proliferating immune cells [5,6]. Numerous studies showed that GLN has immune-modulating effects and is considered an essential amino acid in catabolic conditions [7,8]. Previous studies showed that GLN supplementation attenuated proinflammatory cytokine release, protected against organ damage and decreased mortality in a lipopolysaccharide-treated animal model [9]. Studies performed by our laboratory also found that GLN supplementation produces a more balanced Th1/Th2 response during sepsis [10]. Some investigators suggested that GLN is useful in treating established infections and inflammation. A study by Vicario et al. [11] found that GLN supplementation improved barrier functions in rats with dextran sulfate sodium (DSS)-induced colitis. However, studies concerning the effect of GLN on Th1/Th2/Th17 pathways in IBD are rare.

☆ This study was supported by a research grant (NSC100-2320-038-007-MY3) from the National Science Council, Taipei, Taiwan.
* Corresponding author. Tel.: +886 2 27361661x6547.
E-mail address: sangling@tmu.edu.tw (S.-L. Yeh).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.06.002

Table 1
Histological score of colitis

Feature	Score	Description
Loss of epithelium	0	None
	1	0%–20% loss of epithelium
	2	20%–30% loss of epithelium
	3	Over 30% loss of epithelium
Length of crypts	0	None
	1	Length of crypts was less than 1.5 times of normal thickness
	2	Length of crypts was 1.5–3 times of normal thickness
	3	Length of crypts was 3 or more times of normal thickness
Infiltration of leukocytes	0	None
	1	Mild
	2	Moderate
	3	Severe

According to previous studies, the cytokine profile of acute DSS-induced colitis is consistent with an acute inflammatory response and was found to be characterized by polarization toward the Th1–Th17 panel [12]. We hypothesized that GLN modulates the balance of T-cells and reduces the inflammatory response in acute colitis. Therefore, we pretreated mice with GLN before inducing colitis to investigate whether preventive use of GLN modulates cytokine expressions associated with Th1/Th17 and Th2. Also, systemic and intraluminal inflammatory parameters were measured in the recovery phase of DSS-induced acute colitis.

2. Materials and methods

2.1. Animals

Six-week-old male C57BL/6 mice weighing 22–25 g at the beginning of the experiment were used in this study. All mice were housed in a temperature- and humidity-controlled room and were allowed free access to a standard chow diet for 1 week before the study. Care of laboratory animals was in full compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and protocols were approved by the institutional Animal Care and Use Committee at Taipei Medical University.

2.2. Study protocols

There were one normal control (NC, $n=9$) group and two DSS groups ($n=9$ for each group) in this study. Mice in the NC group received distilled water, whereas the DSS groups drank distilled water containing 3% (wt/vol) DSS (MW 40 kDa; MP Biomedicals, Solon, OH, USA) for 5 days as previously described [13]. One of the DSS groups (G-DSS) was administered a GLN solution (Dipeptiven; Fresenius Kabi, Bad Homburg, Germany), while the other DSS group (C-DSS) received a commercially available mixed amino acid solution without GLN (Moriamin-SN; Chinese Pharmaceuticals, Taipei, Taiwan). The amino acid solution was injected intraperitoneally daily for 3 days before receiving DSS water. The amount of amino acids injected was identical in the two DSS groups. The dosage of GLN was 0.75 g amino acid/kg body weight as alanyl-GLN, which provided 0.5 g GLN/kg/d. This amount of GLN was found to have antioxidant and antiproteolytic properties in a muscular dystrophy mice model [14]. After feeding the DSS water for 5 days, the mice were allowed to recover for 3 days with an intraperitoneal saline injection daily. During the experimental period, body weights were recorded daily. All mice had free access to a standard chow diet and water. At the end of the experiment, mice were anesthetized and sacrificed by cardiac puncture. Fresh blood samples were collected in tubes containing heparin for the analysis of the Th lymphocyte subpopulation distribution. Whole blood was centrifuged at 3000*g* for 10 min at 4°C to obtain plasma. Plasma samples were used to measure haptoglobin (ICL Inc., Newberg, OR, USA). To harvest cells from the peritoneal cavity, a middle abdominal incision was made, and 2 ml of phosphate-buffered saline (PBS) was intraperitoneally injected to elute the peritoneal cells. The peritoneal lavage fluid (PLF) was collected and centrifuged at 300*g* for 10 min. The pellet was used to analyze the population of leukocytes. The colon was cut close to the ileocecal valve, and the length and weight were measured. Luminal content of colons was collected by flushing each specimen with 2 ml of ice-cold PBS. Tissues and colon lavage fluid were kept at −80°C until processed for further analysis.

2.3. Measurements and analytical procedures

2.3.1. Distribution of helper T-lymphocyte subpopulations in blood

To determine the phenotypes of lymphocytes in the blood, 100 μl of whole blood was divided into aliquots and incubated with eFluor 450-conjugated anti-mouse CD4 for 30 min. After lysis of red blood cells, the remaining leukocytes were fixed and permeated for intracellular cytokine staining. Th17-associated cytokines were detected using a Mouse Th17 Cytokine Staining Panel (eBioscience, San Diego, CA, USA) according to the instruction manual. CD4-labeled leukocytes were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IL-17A, phycoerythrin (PE)-conjugated anti-mouse IL-17F and PerCP-eFlour 710-conjugated anti-mouse IL-22 antibodies. To determine populations of Th1 and Th2 cells, FITC-conjugated anti-mouse interferon (IFN)-γ and PE-conjugated anti-mouse IL-4 (Biolegend, San Diego, CA, USA) antibodies were used for incubation with CD4-labeled leukocytes. After incubation for 20 min, cells were washed and suspended in staining buffer and then analyzed with flow cytometry (BD Biosciences, San Jose, CA, USA). CD4-positive cells were gated on the basis of their forward- and side-scatter profiles. The results are presented as a percentage of cytokine-producing cells in CD4-positive cells.

2.3.2. Distribution of leukocyte populations in PLF

Pelleted peritoneal cells were suspended in staining buffer and split into three vials with 100 μl in each vial. Cells in each vial were incubated with allophycocyanin (APC)-conjugated anti-CD45 (Biolegend), a marker of leukocytes. To investigate the distribution of T- and B-cells, FITC-conjugated anti-CD3 and PE-conjugated anti-CD19 antibodies (Biolegend) were added. FITC-conjugated anti-CD4 and PE-conjugated anti-CD8 antibodies (Biolegend) were added when the populations of CD4 and CD8 T-lymphocytes were analyzed. To measure macrophage and neutrophil distributions, Pacific Blue-conjugated anti-CD11b (Biolegend) and PE-conjugated anti-Ly6G antibodies (BD) were used. After incubating the respective vials for 30 min at 4°C in the dark, cells were washed and resuspended in staining buffer to measure the leukocyte population by flow cytometry. Fluorescence data were recorded, and results are presented as a percentage of specific CD-marker-expressing cells in leukocytes.

2.3.3. Immunoglobin (Ig) quantification in colon lavage fluid

Luminal IgA and IgG contents were determined in colon washings. Colon lavage fluid was centrifuged at 2000*g* for 10 min at 4°C, and supernatants were collected for analysis of Ig by an enzyme-linked immunosorbent assay (ELISA) with the corresponding capture antibody for IgA or IgG (ICL, Inc.). The amount of Ig was determined by a detection antibody conjugated to horseradish peroxidase. The protein concentration of the colon lavage fluid was measured by a BioRad Bradford protein assay (Hercules, CA, USA) to adjust the amount of Ig.

2.3.4. Measurements of myeloperoxidase (MPO) activity in the colon

MPO activity was measured using a method modified by Suzuki et al. [15]. Colon samples were homogenized in PBS and centrifuged at 15,000*g* and 4°C for 15 min. The supernatants were collected for further analysis of chemokines, and the pellets were suspended in PBS containing 0.5% hexadecyltrimethylammonium bromide. After freezing and thawing for three cycles, samples were sonicated and centrifuged at 15,000*g* for 15 min at 4°C. Aliquots (30 μl) were added to 470 μl of the reaction mixture containing 40% PBS, 8% *N*,*N*-dimethylformadine, 1.6 mM 3,3′,5,5′-tetramethylbenzidine, 0.3 mM H_2O_2 and 80 mM sodium phosphate (pH 5.4). The absorbance at 655 nm was immediately measured for 3 min at 37°C. The change in absorbance per min was calculated, and the activity of MPO was presented as Δ absorbance per gram of colon tissue.

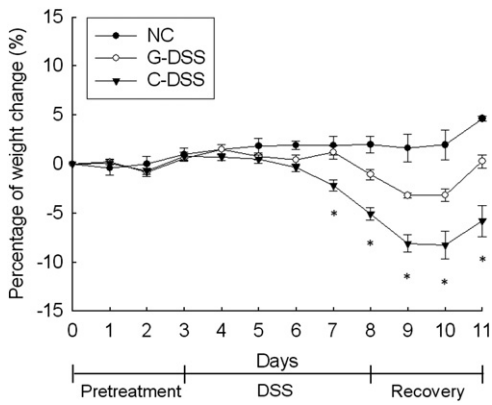

Fig. 1. Percentage of body weight changes of the three groups. Data are presented as the mean±S.E.M. Differences among groups and different time schedules were analyzed by two-way ANOVA using the Bonferroni posttest. *Significantly differs from the C-DSS group ($P<.05$).

2.3.5. Chemokine levels in colon homogenates

Keratinocyte-derived chemokine (KC) and monocyte chemoattractant protein (MCP)-1 levels were measured by ELISA kits (R & D Systems, Minneapolis, MN, USA). Antibodies specific for KC or MCP-1 were coated onto the wells of microtiter strips provided. Procedures followed the manufacturer's instructions. The chemokine concentrations were adjusted by the weight of colon tissue.

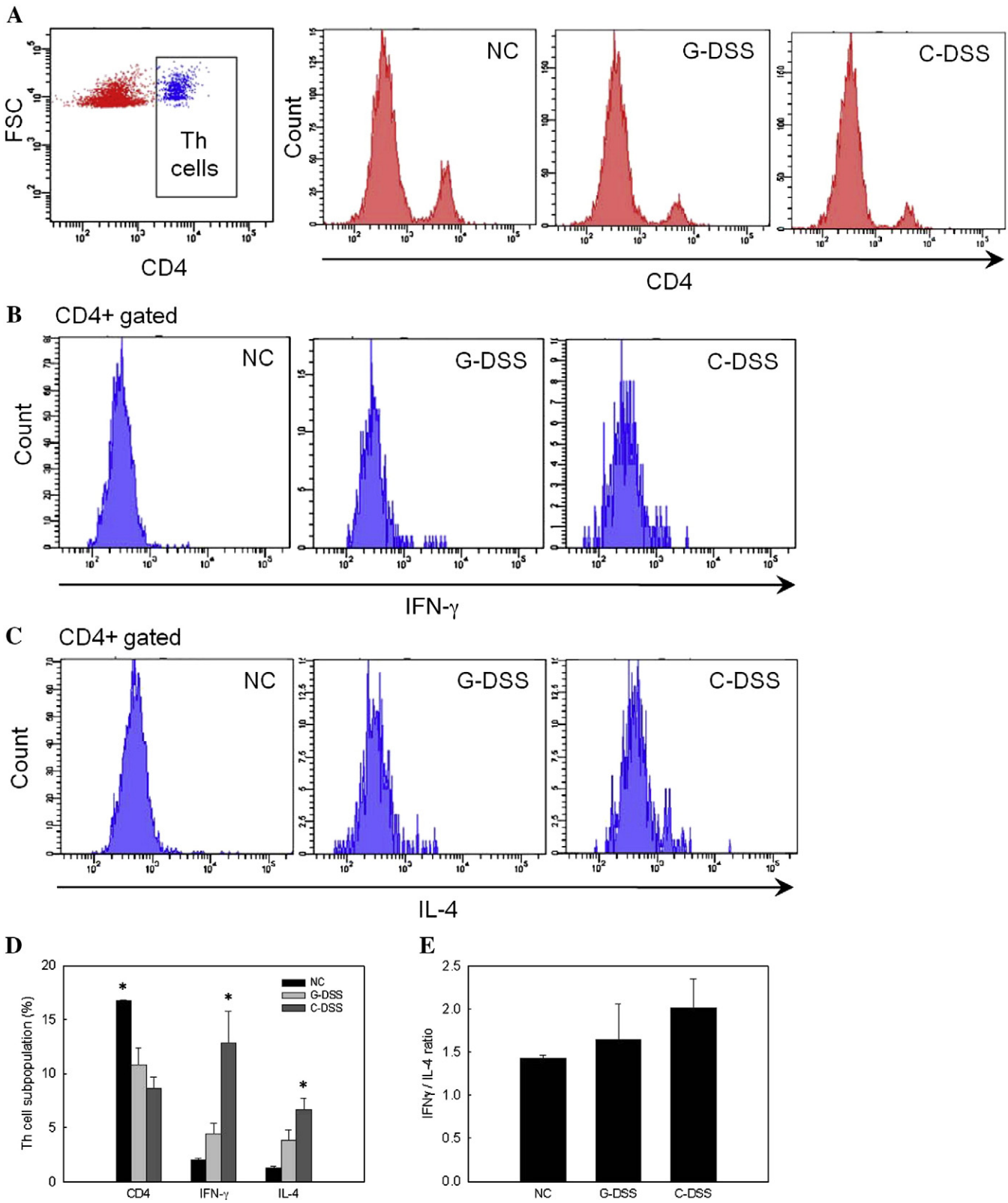

Fig. 2. Percentage of Th cell populations in blood. Blood leukocytes were surface stained for CD4, fixed, stained for intracellular cytokines and analyzed by flow cytometry. CD4-positive cells were considered as Th cells (A) and gated to determine the intracellular IFN-γ and IL-4 expressions (B and C). The percentage of cells in the $CD4^+$ cells that express the indicated cytokines was measured, and data are presented as the mean±S.E.M. (D). The ratio of IFN-γ- and IL-4-expressing Th cells was also calculated (E). Differences among groups were analyzed by one-way ANOVA using the Tukey's test. *Significantly differs from the other two groups (P<.05).

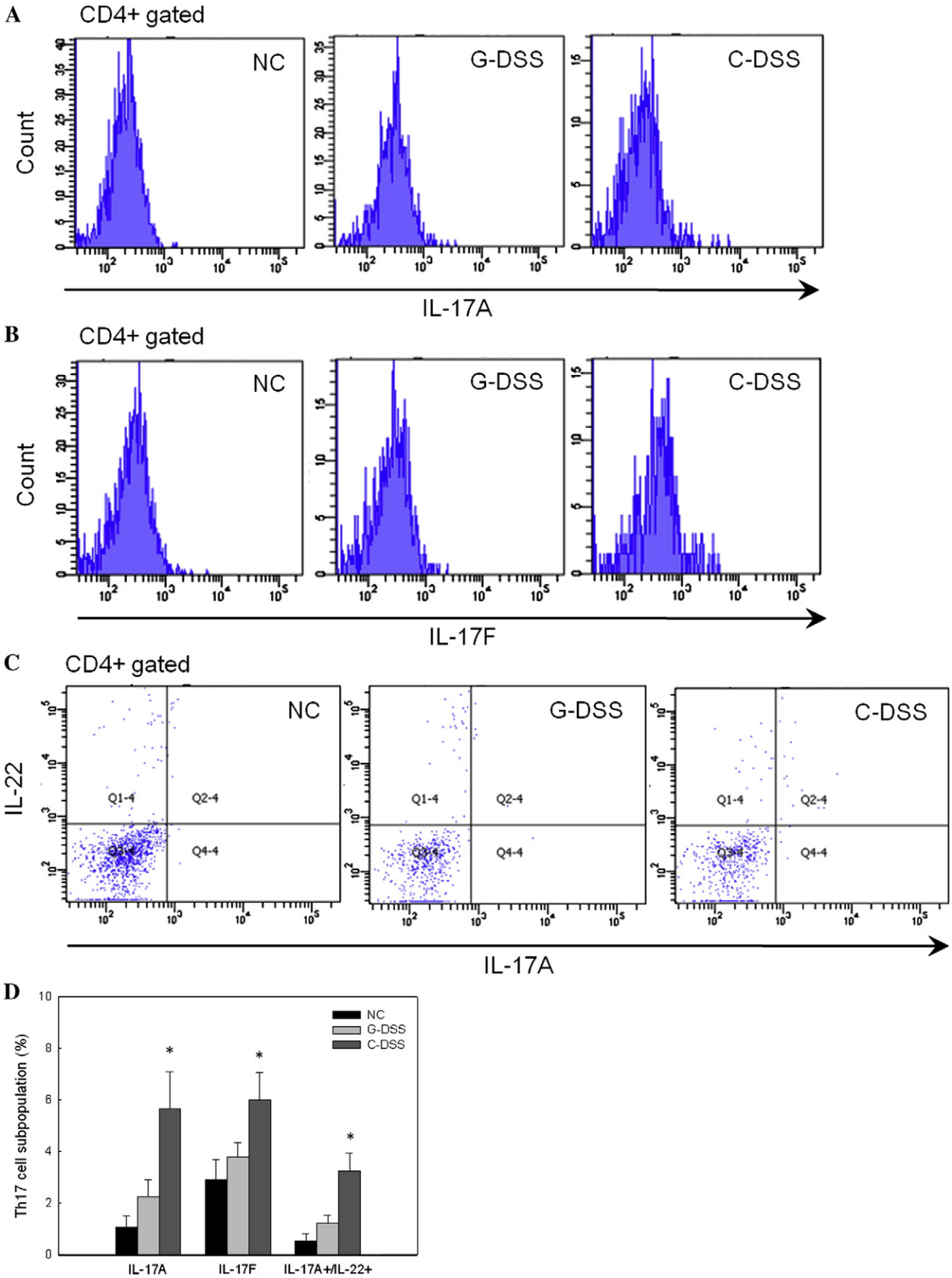

Fig. 3. Percentages of Th17-associated-cytokine-producing cell populations in blood. CD4-positive cells were gated to analyze the intracellular cytokine expressions by flow cytometry. The percentage of Th17-associated-cytokine-expressing cells in $CD4^+$ cells were measured (A, B and C), and values are shown as the mean±S.E.M. (D). Differences among groups were analyzed by one-way ANOVA using the Tukey's test. *Significantly differs from the other two groups ($P<.05$).

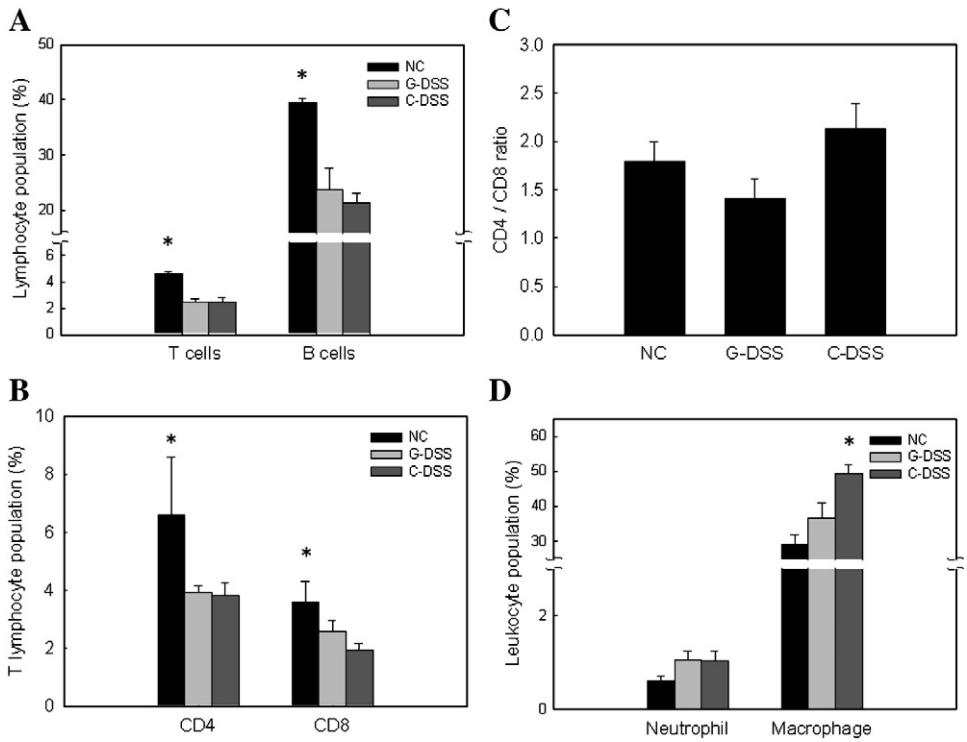

Fig. 4. Distribution of leukocytes in PLF. Pelleted peritoneal cells were surface stained with indicated surface markers and analyzed by flow cytometry. CD45-positive cells were considered as leukocytes and gated to determine the population of leukocyte in PLF. Percentages of T- and B-lymphocytes (A) were determined by CD3- and CD19-expressing cells in $CD45^+$ cells. The subpopulation of T-lymphocytes (B) was analyzed by CD4- and CD8-expressing cells, and the ratio of CD4- and CD8-positive cells (C) was also calculated. Double staining for CD11b and Ly-6G was used to identify macrophages ($CD11b^+$/Ly-$6G^-$) and neutrophils ($CD11b^+$/Ly-$6G^+$) populations (D). Values are presented as the mean±S.E.M. Differences among groups were analyzed by one-way ANOVA using the Tukey's test. *Significantly differs from the other two groups (P<.05).

2.3.6. Histopathology

Sections (1 cm) of the distal and proximal colon were cut out. The middle segment of colon tissues was collected and fixed with buffered 4% paraformaldehyde. After being embedded in paraffin, specimens were sectioned at 5 μm, mounted on glass slides and stained with hematoxylin and eosin for the histopathology analysis. Digital images at 40× magnification per section were captured by a Zeiss Axiphot light microscope (Carl Zeiss MicroImaging LLC, Thornwood, NY, USA) and a Nikon D1X digital camera (Tokyo, Japan). Five fields per section were examined to determine the morphological lesions and changes of the colon mucosa. The degree of IBDs was measured by the modified scoring system of Iba et al. [16]. The inflammatory colitis was scored from 0 to 3 for lesions based on loss of epithelium, length of crypts and infiltration of leukocytes (Table 1). The total histological score ranged from 0 to 9, which represented the sum scores of loss of epithelium, length of crypts and infiltration of leukocytes.

2.4. Statistical analysis

All data are expressed as the mean±standard error of the mean (S.E.M.). Differences among groups were analyzed by analysis of variance (ANOVA) using the Tukey's test. Two-way ANOVA using the Bonferroni posttest was used to analyze the differences of body weight changes. A P value of <.05 was considered statistically significant.

3. Results

3.1. Weight change

There were no differences in initial body weights among the three groups. Clinical symptoms of exposure to DSS for 5 days (days 3–8 of the experimental period) were loss of body weight and fecal blood. Body weight loss was observed 4 days (day 7) after drinking the DSS water and reached a peak 2 days (day 10) after exposure to DSS was stopped. Weight loss was less in the G-DSS group than in the C-DSS group at 7–11 days (Fig. 1).

3.2. Cytokine expressions in blood CD4 cells

The CD4 percentage was lower in the DSS groups than the NC group. The percentages of IL-17A, IL-17F, IL-17A+/IL-22+, IL-4 and IFN-γ were significantly lower in the G-DSS group than the C-DSS group and showed no differences from the NC group (Figs. 2, 3). There were no differences in the IFN-γ/IL4 ratio among the three groups.

3.3. Plasma haptoglobin levels

Haptoglobin levels were higher in the DSS-treated groups than the NC group (6.75±3.05 vs. 853.0±74.5 μg/ml, P<.0001). The level in the G-DSS group was significantly lower than that in the C-DSS group (617.8±75.9 vs. 1089.2±46.2 μg/ml, P<.01).

3.4. Leukocyte expressions in PLF

Both DSS groups had lower percentages of B, T, CD4 and CD8 cells than the NC group. There were no differences in these parameters between the two DSS groups. The percentage of macrophages ($CD11b^+$/Ly-$6G^-$) was significantly higher in the C-DSS group than in the G-DSS and NC groups. There were no differences in neutrophil ($CD11b^+$/Ly-$6G^+$) expressions in the PLF among the three groups (Fig. 4).

3.5. Colon length, Ig, chemokine levels and MPO activities

The length of the colon was significantly shorter in the C-DSS group than the other two groups (Table 2). IgA levels in the colon lavage fluid did not differ among the three groups, whereas IgG levels were significantly higher in the DSS groups than the NC group (Table 3). Also, KC and MCP-1 levels as well as MPO activities in

Table 2
Length and weight of colons

	NC	G-DSS	C-DSS
Length (cm)	6.85±0.27	6.07±0.13	5.59±0.21[a]
Weight (g)	0.504±0.041	0.526±0.025	0.544±0.035
Weight/length (g/cm)	0.072±0.004	0.083±0.004[a]	0.101±0.05[a,b]

Data are expressed as the mean±S.E.M. Differences among groups were analyzed by ANOVA using the Tukey's test.
[a] Significantly differs from the NC group.
[b] Significantly differs from the G-DSS group ($P<.05$).

colon homogenates were significantly higher in the C-DSS group than in the NC and G-DSS groups. The G-DSS group had lower IgG, KC and MCP-1 levels than the C-DSS group (Table 3).

3.6. Histopathological aspects of the colon

Histological observations showed that colon tissues of the NC group had intact epithelium, well-defined gland length and no leukocyte infiltration in the mucosa (Fig. 5A). DSS-induced colitis resulted in mucosal ulceration, infiltration of leukocytes, crypt distortion and hyperplastic epithelium. Compared to the G-DSS group, more severe inflammatory lesions of the colic mucosa were observed in the C-DSS group (Fig. 5B, C). Histological evaluation of inflammatory score is shown in Fig. 5D. The G-DSS group had a lower score than the C-DSS group.

4. Discussion

IBD is a disorder that flares up with remissions and relapses. Animal models of IBD have significantly contributed to our present understanding of the disease, as they provide a platform through which some of these complex mechanisms can be systemically investigated. DSS is a physical agent with an intrinsic capacity to disrupt the epithelial cell barrier and activate mucosal macrophages, which in turn produce immunomodulatory cytokines [17]. Dieleman and colleagues [18] demonstrated that adaptive immune system is not required for development of DSS-induced colitis in severe combined immunodeficient mice. However, Shintani et al. [19] found that $CD4^+$ T (Th) cells are responsible for the modulation of DSS-induced colitis. Moreover, Melgar et al. [13] indicated that acute colitis induced by DSS progressed to chronicity in C57BL/6 mice. After DSS removal, IFN-γ and IL-17 in colon homogenates and infiltration of immune cells into colon tissue were progressively up-regulated leading to chronic colitis, suggesting that activated T-cells are involved in progression of DSS-induced colitis. In this study, we administered DSS orally to C57BL/6 mice because animals in this model were found to develop acute colitis that progressed to severe chronic inflammation resembling UC [13]. We pretreated animals with GLN for 3 days before colitis was induced. This model may be valid for the acute phase of UC in patients, in whom preventive administration of GLN may be recommended.

A cascade of inflammatory mediators, primarily cytokines, represents key players of the innate and adaptive immune response. Under normal conditions, the intestinal mucosa functions with a delicate balance of inflammatory cells for which cytokine-induced signals are tightly regulated by intricate feedback mechanisms[12]. In IBD, the pathophysiologically dysregulated immunologic response is reflected by an imbalance in the cytokine production profile at different stages of the disease process [12]. This dysregulated imbalance is thought to be represented by a Th1/Th2/Th17 polarization pattern in IBD [20]. Th1 cells produce large quantities of IFN-γ, while Th2 cells produce IL-4 and IL-10. Th17 effector functions are distinct from Th1- and Th2-mediated immunity. Th17 cells produce IL-17A, IL17F, IL-21 and IL-22 [21]. By producing cytokines and attracting highly specialized effector cells like neutrophils and macrophages, Th17 cells appear to be critical for enhancing host protection against tissue-infiltrating pathogens. On the other hand, this also correlates with a high potency to initiate substantial immunology and autoimmune diseases [22]. Dysregulation in either Th direction can result in intestinal inflammation.

In this study, we observed that DSS-induced colitis resulted in immune suppression because blood CD4 cells together with T- and B-cells in the PLF were lower than those in control mice. A previous study showed that acute DSS-induced colitis demonstrated a cytotoxic and chemotactic profile with significantly elevated levels of IL-6, IL-17, tumor necrosis factor-α and KCs when compared to controls. This cytokine profile is characterized by a polarized Th1–Th17 panel [12]. In this study, we analyzed Th17-associated cytokines including IL-17A, IL-17F and IL-17A+/IL-22+. IL-22 plays critical roles in inflammation, immune surveillance and tissue homeostasis at mucosal sites [23]. Because IL-22 is produced by immune cells other than Th17 cells [24], we measured IL-17A+/IL-22+-presenting cells to ensure that IL-22 was derived from Th17 cells. Our results were consistent with previous findings that Th17-associated cytokines and Th1-associated IFN-γ were higher in the C-DSS group [12]. In addition, we also found that IL-4 expressions were higher in the DSS colitis groups, and thus, the IFN-γ/IL-4 ratio showed no differences among the normal and DSS groups. This finding was comparable to a report by Dieleman et al. [25], who also found increased splenic IFN-γ- and IL-4-presenting cells after cessation of DSS consumption. This phenomenon could be due to a slow recovery after DSS damage followed by nonspecific immune activation in which both Th1 and Th2 cytokines play a role [25]. A recent report by Sonnenberg et al. [24] showed that IL-17A may regulate the expression and/or proinflammatory properties of IL-22. Coexpressions of IL-17A and IL-22 promote tissue damage and inflammation [23]. In this study, we found that pretreatment with GLN produced lower Th1/Th2/Th17 cytokine expressions in the recovery phase of acute DSS-induced colitis. This finding might mean that inflammation mediated by populations of Th cells was attenuated when GLN was administered.

Haptoglobin is an acute-phase protein. It is used as a marker of systemic inflammation to monitor disease activity in experimental colitis in murine models [13]. IgG concentration in gut lavage fluid is a specific index for grading disease activity in patients with IBD [26]. A previous study showed that intestinal lymphocytes of patients with active IBD produce high levels of IgG [27]. In this study, we found that DSS administration resulted in higher plasma haptoglobin and intraluminal IgG, indicating that experimental colitis was active. Pretreatment with GLN lowered IgG levels, suggesting that the severity of DSS-induced colitis was attenuated when GLN was administered. In contrast to IgG, IgA levels did not differ among the NC and the DSS colitis groups. This result is consistent with a report by Macpherson et al. [27], who also found

Table 3
Colon Ig concentrations in lavage fluid and MPO activities and chemokines concentrations in homogenates

	NC	G-DSS	C-DSS
Colon lavage fluid			
IgA (μg/mg protein)	9.78±3.56	4.68±1.76	7.07±1.82
IgG (μg/mg protein)	3.95±0.97	8.38±1.66[a]	22.92±4.19[a,b]
Colon homogenates			
MPO (ΔABS/min/g tissue)	0.957±0.030	1.067±0.074	1.405±0.089[a]
KC (ng/g tissue)	ND	3.00±0.85[a]	40.22±17.33[a,b]
MCP-1(ng/g tissue)	0.48±0.16	6.12±0.87[a]	14.27±2.69[a,b]

Data are expressed as the mean±S.E.M. Differences among groups were analyzed by ANOVA using the Tukey's test. ND: not detectable; MPO, myeloperoxidase.
[a] Significantly differs from the NC group.
[b] Significantly differs from the G-DSS group ($P<.05$).

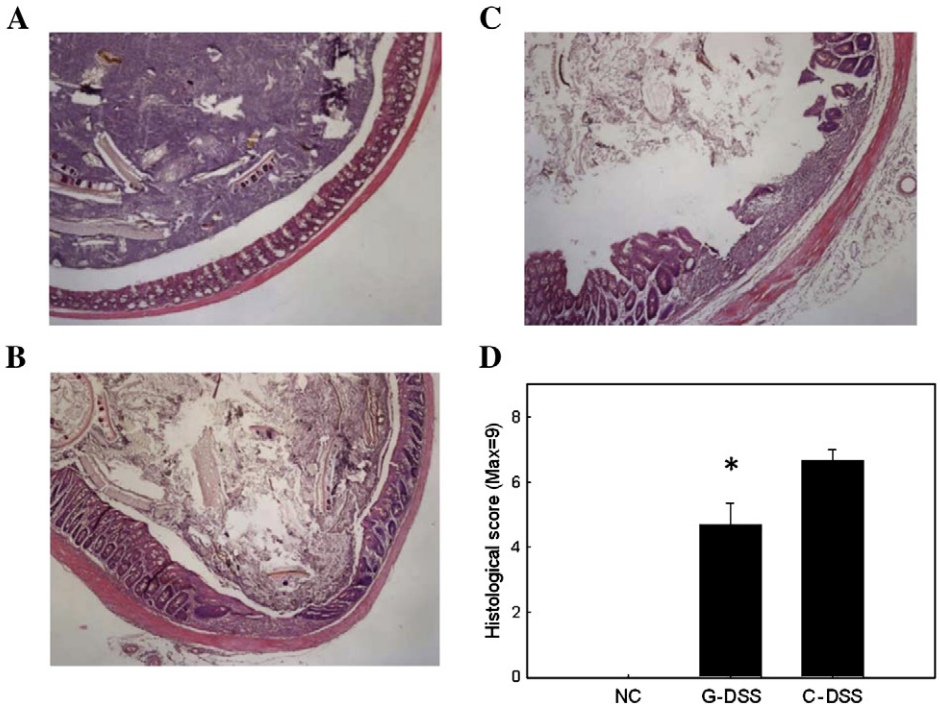

Fig. 5. Histopathology of colon tissues. Hematoxylin and eosin staining of colon tissues from mice in the control group (A), G-DSS group (B) and C-DSS group (C). Representative histological images of normal and DSS-induced mice are shown at 40× magnification. Normal colon section showed intact epithelium, a well-defined gland and no leukocyte infiltration in the mucosa. In contrast, mucosal ulceration, gland distortion and leukocyte infiltration were obvious in both DSS-induced colitis groups. Mucosal inflammation of the C-DSS group was more severe compared to that of the G-DSS group. Histological scores of colitis (D) are presented as the mean±S.E.M., which were determined as described in "Materials and Methods." Differences among groups were analyzed by one-way ANOVA using the Tukey's test. *Significantly differs from the other two groups ($P<.05$).

that total mucosal IgA concentrations did not differ between an IBD group and controls.

MPO is an enzyme synthesized by neutrophils and monocyte precursor cells. MPO plays an important role in leukocyte-mediated tissue injury responses in inflammatory diseases [28]. A previous study found that clinical and endoscopic activities correlated well with intraluminal MPO concentrations in UC patients [29]. Because oxyradicals released from leukocytes that accumulate in organs may damage organ cells and induce organ dysfunction [28], we analyzed MPO activities in the colon as an indicator for identifying the extent of tissue injury resulting from colitis. We found that DSS resulted in high MPO activities, indicating that more leukocytes had infiltrated into colon tissues, and GLN administration reversed this inflammatory condition. This result was consistent with a lower percentage of macrophage presenting in the abdomen and lower KC and MCP-1 in colon homogenates in the G-DSS group. KC is a chemokine that plays an important role in mediating neutrophil infiltration to tissues, and MCP-1 is a chemotactic and activating factor for mononuclear phagocytes. The lower Th17 cytokine expressions observed in the G-DSS group attracted fewer leukocytes to the site of inflammation and attenuated damage to the colon. Colon length and histological findings also confirmed that the severity of the inflammatory lesions was lower in the G-DSS group.

A previous study showed that GLN supplementation reduced intestinal luminal water contents during mild inflammation and improved the barrier function in rats with DSS-induced colitis [11]. Oral GLN prevents gut mucosal injury and improves mucosal recovery after lipopolysaccharide endotoxemia in rats [30]. GLN is the precursor for the synthesis of glutathione (GSH). GLN was found to be rate limiting for GSH synthesis, and the availability of GLN is critical for generating GSH stores [31]. Yu et al. [32] found that GLN-supplemented nutrition preserved GSH stores after treatment with an antineoplastic agent (5-fluorouracil). An *in vitro* study by Babu et al. [33] also found that GLN prevented damage to the liver, and this was possibly mediated by GSH synthesis. It is possible that GLN decreased oxidative stress induced by colitis, which may consequently have reduced the expressions of Th17 cytokines and decreased the production of inflammatory mediators. The mechanism responsible for the effect of GLN on colitis is under investigation in our laboratory.

In summary, this study showed for the first time that pretreatment with GLN reduced plasma haptoglobin and suppressed Th1/Th17/Th2 cytokine production. Also, luminal IgG and colon chemokines levels and MPO activities were lower in the G-DSS group. This result implies that pretreatment with GLN suppressed Th-cell-associated cytokine expressions, reduced inflammatory markers, decreased leukocyte infiltration to tissues and may consequently have ameliorated the severity of acute DSS-induced colitis.

References

[1] Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010;28:573–621.
[2] Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Annu Rev Immunol 2002;20:495–549.
[3] Caprioli F, Pallone F, Monteleone G. Th17 immune response in IBD: a new pathogenic mechanism. J Crohns Colitis 2008;2:291–5.
[4] Abraham C, Cho JH. IL-23 and autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. Annu Rev Med 2009;60:97–110.
[5] Wilmore DW, Shabert JK. Role of glutamine in immunologic responses. Nutrition 1998;14:618–26.
[6] Souba WW. Glutamine: a key substrate for the splanchnic bed. Annu Rev Nutr 1991;11:285–308.
[7] Parry-Billings M, Evans J, Calder PC, Newsholme EA. Does glutamine contribute to immunosuppression after major burns? Lancet 1990;336:523–5.
[8] Wilmore DW. The effect of glutamine supplementation in patients following elective surgery and accidental injury. J Nutr 2001;131:2543S–99S [discussion 2550S-1S].
[9] Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. Shock 2001;16:398–402.

[10] Yeh CL, Hsu CS, Yeh SL, Chen WJ. Dietary glutamine supplementation modulates Th1/Th2 cytokine and interleukin-6 expressions in septic mice. Cytokine 2005;31:329–34.
[11] Vicario M, Amat C, Rivero M, Moreto M, Pelegri C. Dietary glutamine affects mucosal functions in rats with mild DSS-induced colitis. J Nutr 2007;137:1931–7.
[12] Alex P, Zachos NC, Nguyen T, Gonzales L, Chen TE, Conklin LS, et al. Distinct cytokine patterns identified from multiplex profiles of murine DSS and TNBS-induced colitis. Inflamm Bowel Dis 2009;15:341–52.
[13] Melgar S, Karlsson A, Michaelsson E. Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Am J Physiol Gastrointest Liver Physiol 2005;288:G1328–38.
[14] Mok E, Constantin B, Favreau F, Neveux N, Magaud C, Delwail A, et al. l-Glutamine administration reduces oxidized glutathione and MAP kinase signaling in dystrophic muscle of mdx mice. Pediatr Res 2008;63:268–73.
[15] Suzuki K, Ota H, Sasagawa S, Sakatani T, Fujikura T. Assay method for myeloperoxidase in human polymorphonuclear leukocytes. Anal Biochem 1983;132:345–52.
[16] Iba Y, Sugimoto Y, Kamei C, Masukawa T. Possible role of mucosal mast cells in the recovery process of colitis induced by dextran sulfate sodium in rats. Int Immunopharmacol 2003;3:485–91.
[17] Kitajima S, Takuma S, Morimoto M. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Exp Anim 1999;48:137–43.
[18] Dieleman LA, Ridwan BU, Tennyson GS, Beagley KW, Bucy RP, Elson CO. Dextran sulfate sodium-induced colitis occurs in severe combined immunodeficient mice. Gastroenterology 1994;107:1643–52.
[19] Shintani N, Nakajima T, Okamoto T, Kondo T, Nakamura N, Mayumi T. Involvement of CD4+ T cells in the development of dextran sulfate sodium-induced experimental colitis and suppressive effect of IgG on their action. Gen Pharmacol 1998;31:477–81.
[20] Fuss IJ, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, et al. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. J Immunol 1996;157:1261–70.
[21] Costa VS, Mattana TC, da Silva ME. Unregulated IL-23/IL-17 immune response in autoimmune diseases. Diabetes Res Clin Pract 2010;88:222–6.
[22] Korn T, Oukka M, Kuchroo V, Bettelli E. Th17 cells: effector T cells with inflammatory properties. Semin Immunol 2007;19:362–71.
[23] Colonna M. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. Immunity 2009;31:15–23.
[24] Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. J Exp Med 2010;207:1293–305.
[25] Dieleman LA, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG, et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol 1998;114:385–91.
[26] Choudari CP, O'Mahony S, Brydon G, Mwantembe O, Ferguson A. Gut lavage fluid protein concentrations: objective measures of disease activity in inflammatory bowel disease. Gastroenterology 1993;104:1064–71.
[27] Macpherson A, Khoo UY, Forgacs I, Philpott-Howard J, Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut 1996;38:365–75.
[28] Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005;77:598–625.
[29] Sangfelt P, Carlson M, Thorn M, Loof L, Raab Y. Neutrophil and eosinophil granule proteins as markers of response to local prednisolone treatment in distal ulcerative colitis and proctitis. Am J Gastroenterol 2001;96:1085–90.
[30] Sukhotnik I, Agam M, Shamir R, Shehadeh N, Lurie M, Coran AG, et al. Oral glutamine prevents gut mucosal injury and improves mucosal recovery following lipopolysaccharide endotoxemia in a rat. J Surg Res 2007;143:379–84.
[31] Welbourne TC. Ammonia production and glutamine incorporation into glutathione in the functioning rat kidney. Can J Biochem 1979;57:233–7.
[32] Yu JC, Jiang ZM, Li DM, Yang NF, M-X B. Alanyl-glutamine preserves hepatic glutathione stores after 5-FU treatment. Clin Nutr 1996;15:261–5.
[33] Babu R, Eaton S, Drake DP, Spitz L, Pierro A. Glutamine and glutathione counteract the inhibitory effects of mediators of sepsis in neonatal hepatocytes. J Pediatr Surg 2001;36:282–6.