Article

Inhibitory Effect of Recombinant IL-25 on the Development of Dextran Sulfate Sodium-Induced Experimental Colitis in Mice

S.S. Salum Mchenga[1], Danan Wang[1], Cheng Li[1], Fengping Shan[1] and Changlong Lu[1, 2]

The role of interleukin 25 (IL-25) in a number of human diseases still has not been extensively studied, here we attempt to evaluate the role of recombinant IL-25 (rIL-25) in the development of dextran sulfate sodium (DSS)-induced experimental colitis. Acute colitis was induced in female C57BL/6 mice by oral administration of 2.5% DSS in drinking water *ad libitum*. At the same time as the start of DSS exposure, mice were injected intraperitoneally with 0.4 μg of rIL-25 or PBS. Then disease activity index (DAI), histological changes and survival rate were observed. The levels of IL-17, IL-23, and TGF-β1 in colon tissues were determined by ELISA, and the production of IL-17 by $CD4^+/CD8^+$ T cells was detected by intracellular flow cytometry. In contrast to the DSS treated mice, DSS + rIL-25 treated mice displayed a lower DAI, limited histological changes and prolonged survival. The levels of IL-23 and TGF-β1 were significantly elevated in the DSS + rIL-25 treated mice compared to the DSS treated mice. There was no significant difference in the production of IL-17 in colon tissues and $CD4^+/CD8^+$ T cells between the DSS + rIL-25 treated mice and DSS treated mice. Our findings suggest the role of IL-25 in inhibiting development and progression of acute colitis in DSS-induced mouse colitis model. *Cellular & Molecular Immunology*. 2008;5(6):425-431.

Key Words: IL-25, dextran sulfate sodium, disease activity index, colitis

Introduction

Inflammatory bowel diseases (IBDs) such as Crohn's disease and ulcerative colitis are unknown etiology gastrointestinal disorders associated with chronic relapsing inflammation (1). Histologically, IBDs were characterized by inflammation of the intestinal mucosa with massive infiltration by inflammatory cells (2). Ulcerative colitis was reported to show cytokine profile of Th2 response and to only affect the part of large intestine (3). DSS-induced experimental colitis model is still regarded as a reliable model for the IBD cellular and molecular studies (4). It has been reported that the Th1 cytokine response and histological changes in acute DSS-induced colitis model resemble those observed in human IBDs (5).

IL-25 (IL-17E) is a member of a recently emerged cytokine IL-17 family that comprises six members, IL-17A, B, C, D, E, and F with a protein homology between 20~50% (6). Structurally, all family members have been described to possess cystine residues that account for the characteristic formation of cystine-knot (7). However, IL-25 biological activities appear to markedly differ from those shown by its fellow members as it favors promotion of Th2-associated cytokines (8). IL-25 is reported to be produced by Th2 and mast cells (8, 9). IL-25 was also shown to induce production of pro-inflammatory chemokine IL-8 and to activate NF-κB1 (10). Initially, treatment of mice with rIL-25 was shown to induce massive histological and pathological changes in the gastrointestinal (GI) tract (8). Lately, in parasitic intestinal infectious disease models, IL-25 was reported not only to limit production of pro-inflammatory cytokines but also to reduce chronic intestinal inflammation (11, 12). In light of these earlier studies, we sought to evaluate the effect of rIL-25 on the development of acute DSS-induced experimental colitis.

Materials and Methods

Reagents

DSS (molecular weight 36,000-50,000) was purchased from

[1]Department of Immunology, College of Basic Medical Science, China Medical University, Shenyang 110001, China;

[2]Corresponding to: Dr. Changlong Lu, Department of Immunology, College of Basic Medical Science, China Medical University, 92 North Second Road, Heping District, Shenyang 110001, China. Tel: +86-24-2325-6666 Ext.5346, Fax: +86-24-2325-5291, E-mail: cllu@mail.cmu.edu.cn

Received Aug 2, 2008. Accepted Dec 15, 2008.

Copyright © 2008 by The Chinese Society of Immunology

Abbreviations: rIL-25, recombinant interleukin 25; DSS, dextran sulfate sodium; DAI, disease activity index; IBD, inflammatory bowel disease; i.p., intraperitoneal.

MP Biomedical ICN (France). Recombinant mouse IL-25 was purchased from R&D System (Minneapolis, USA). IL-17, IL-23 and TGF-β1 ELISA Kits were from eBioscience Inc. (San Diego, CA, USA). FITC-conjugated rat anti-mouse CD4, PE-Cy5 rat anti-mouse CD8, PE rat anti-mouse IL-17, purified rat anti-mouse CD16/CD32 (mouse Fc Block) and BD Cytofix/Cytoperm Fixation/Permiabilization Kit were from BD Bioscience (San Jose, USA). Phorbol-12-myristate-13-acetate (PMA) and ionomycin from Sigma-Aldrich (USA), and protease cocktail inhibitor were from AMRESCO Inc. (USA).

Animals

Female C57BL/6 mice aged 6-8 weeks were purchased from Shanghai Experiment Center of Chinese Academy of Science, China. They were maintained at 22°C under 12-h day/night cycle throughout experiment. The study protocol was approved by the Ethical and Research Committee of China Medical University and materials relating to animal experiment conform to the standards currently applied in China.

Induction of colitis and rIL-25 i.p. treatment

Acute experimental colitis was induced by administration of DSS for 5 days. The mice weighing between 18-22 g were randomly divided into three groups. For the DSS + rIL-25 treated group, mice were orally administered with 2.5% (wt/vol) DSS in drinking water, at the same time were treated *i.p.* with 0.4 μg of rIL-25 in PBS (per mouse) daily. For the DSS treated group, mice were orally administered with 2.5% (wt/vol) DSS in their drinking water as were treated *i.p.* with PBS only, and for the control group, mice received tap water. Six mice from each group were sacrificed at day 6 and tissues were harvested for analysis. For survival study, 10 mice from DSS + rIL-25 treated group and DSS treated group were further subjected to DSS until day 7 when they were returned to normal drinking water while assessed for their survival.

Clinical and macroscopic assessment of colitis

Clinical assessment of DSS-induced colitis was performed in all animals daily. Three parameters were the basis of our judgment, including body weight, stool consistency, and the presence of blood in stool. These clinical features were separately scored (Table 1), combined, and then divided by three used parameters to determine disease activity index (DAI) in each group. At autopsy, macroscopic assessment of colitis was performed by measuring colon length and assessing inflammatory changes including edema, hemorrhage, and hyperemia. In brief, at day 6, the mice from all groups were sacrificed through cervical dislocation, their colons were dissected and the length from the ileocecal junction to the anal verge was measured and recorded. The tissues were further processed for histological and cytokine analysis.

Histological assessment of colitis

Samples of the distal colon from each mouse were fixed in 4% paraformaldehyde, processed and embedded in paraffin. Tissue sections of 5 μm thick were cut and stained with hematoxylin and eosin. Histological evaluation was performed in a blinded fashion using a validated scoring system described in detail by Cooper et al. (13): grade 0, normal colonic mucosa; grade 1, shortening and loss of the basal one-third of crypts with mild inflammation and edema in the mucosa; grade 2, loss of the basal two-thirds of the crypts with moderate inflammation in the mucosa; grade 3, loss of entire crypts with severe inflammation in the mucosa with remaining surface epithelium; grade 4, the loss of entire crypts and surface epithelium with massive inflammation in the mucosa, muscularis propria, and sub mucosa.

Table 1. Scoring system for DAI

Score	Weight loss	Stool consistency*	Blood stool
0	none	normal	no blood
1	1~5%	slightly loose	slightly bloody
2	6~10%	loose stools	bloody
3	>10%	diarrhea	gross bleeding[#]

*Stool consistency: normal stool, stool with an appearance of well formed pellets; slightly loose stool, a stool with any absence of well formed pellets; loose stool, stool with pasty, semi formed, soft materials that not adhere to anal fur; diarrhea, liquid stool that adhere to anal fur.
[#]Gross bleeding, an appearance of visible blood that adheres to anal fur.

Cytokine assay

Murine IL-17, IL-23 and TGF-β1 cytokines were analyzed by ELISA on colon tissue homogenates according to manufacturer's instructions. Briefly, the distal colons from each group were collected and homogenized with PBS homogenizing buffer containing 1% Triton X-100 supplemented with cocktail of protease inhibitors. The homogenized solutions were centrifuged at 12,000 rpm for 10 min, and the supernatants were separated into aliquots and stored at -70°C.

Isolation of splenocytes

The spleens from each treatment group were aseptically removed, placed in 100-mm tissue culture dishes, flushed with PBS supplemented with 2% fetal calf serum (2% FCS PBS) using a 5-ml syringe, and then cells were centrifuged to a pellet at 1,500 rpm. Red blood cells were lysed from spleen samples by incubating cell suspensions for 5 min at 4°C in erythrocyte lysis buffer (170 mM Tris, 160 mM NH_4Cl, pH 7.4), followed by two washes in 2% FCS PBS at 1,500 rpm. The supernatants were discarded and the cells were resuspended in RPMI 1640 supplemented with 10% FCS. The cells were counted with a hemocytometer, adjusted at 2×10^6 and then cultured in RPMI 1640 at 37°C in a humidified incubator with 5% CO_2.

Intracellular flow cytometry

Intracellular cytokine staining was performed to assess the production of IL-17 by splenocytes. Cells were previously

Table 2. Clinical presentation of DSS-induced colitis parameters at day 6

Group (n = 6)	Weight loss (% body weight)	Percentage of animals				DAI
		Loose stools	Diarrhea	Blood stools	Gross bleeding	
DSS treated	14	0	100	100	66.6	2.7 ± 0.25*
DSS + rIL-25	13	16.6	66.6	100	16.6	2.0 ± 0.16
Control	0	0	0	0	0	0.0 ± 0.00

The DSS treated mice exhibited much severer clinical symptoms than that in the DSS + rIL-25 treated mice. The DAI data represent mean ± SD (n = 6/group), *$p < 0.05$.

stimulated with 50 ng/ml PMA and 750 ng/ml ionomycin in the presence of Brefeldin A 10 μg/ml, for 4.5 h at 37°C in a humidified incubator with 5% CO_2. To block Fc receptors, cells were preincubated with 1 μg BD Fc block reagent, for 15 min at 4°C. Cells were harvested and directly stained with surface markers anti-CD4-FITC (RM4-5) and anti-CD8-PE-Cy5 for 30 min at 4°C, followed by two washes in staining buffer. Cells were pellet by centrifugation at 2,000 rpm and then resuspended. To proceed with intracellular staining, cells were firstly fixed and permiabilized with BD Cytofix/Cytoperm solution for 20 min at 4°C. Thereafter, cells were washed twice with BD Perm/Wash buffer and then were stained intracellularly with anti-IL-17-PE (TC11-18H10.1) for 30 min at 4°C. Cells were pellet, resuspended in staining buffer and analyzed by flow cytometry.

Statistics

Using SPSS software, the values were expressed as mean ± SD. Continuous data from three groups were evaluated using analysis of variance (ANOVA). In the presence of significant F values, individual comparisons between means were made using the Student-Newman-Keuls (SNK) test. Student's unpaired t test was used to compare difference between two groups. Survival study was analyzed using the Kaplan-Meier log-rank test. The value of $p < 0.05$ was considered as statistically significant.

Results

DSS + rIL-25 treated mice displayed delayed and less severe clinical symptoms

For clinical assessment, DAI was determined to express the severity of colitis. DAI was found to be lower in the DSS + rIL-25 treated mice than in the DSS treated mice ($p < 0.05$). DSS treated mice began to develop colitis at day 3 of DSS exposure and gradually started displaying changes in body weight and stool consistency. Loose stools with the presence

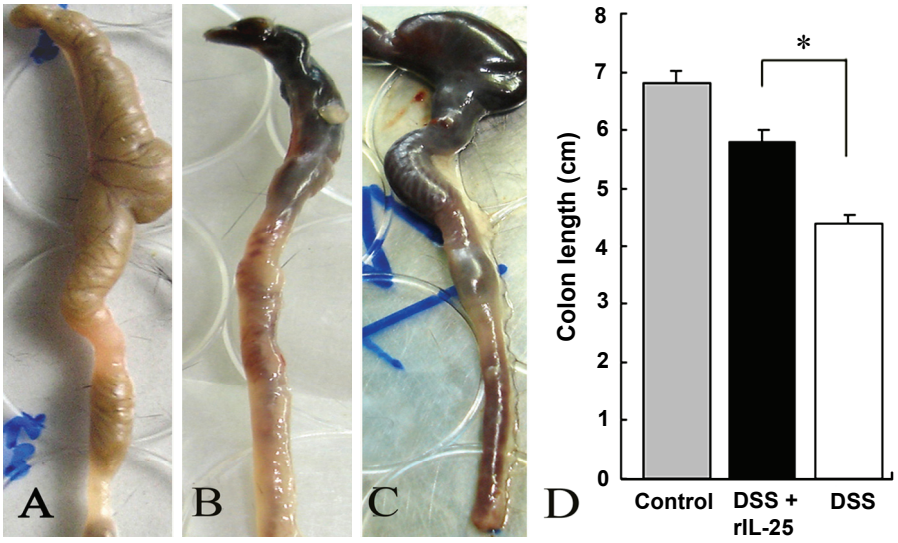

Figure 1. Colons gross appearances showed DSS + rIL-25 treated mice exhibited less clinical symptoms. Mice were divided into three groups. (A) In the control group, mice were given tap water. (B) In the DSS + rIL-25 treated group, mice were orally administered with 2.5% DSS in their drinking water and daily treated *i.p.* with 0.1 ml of 0.4 μg rIL-25 in PBS. (C) For the DSS treated group, mice were orally administered with 2.5% DSS in their drinking water and daily treated *i.p.* with 0.1 ml PBS only. These mice exhibited a severe edema, hemorrhage, hyperemia, colon shortening, and the loss of pellet formation. (D) Colon length statistics of the control, DSS + rIL-25 and DSS treated mice. The data present means ± SD (n = 6 mice/group), *$p < 0.05$.

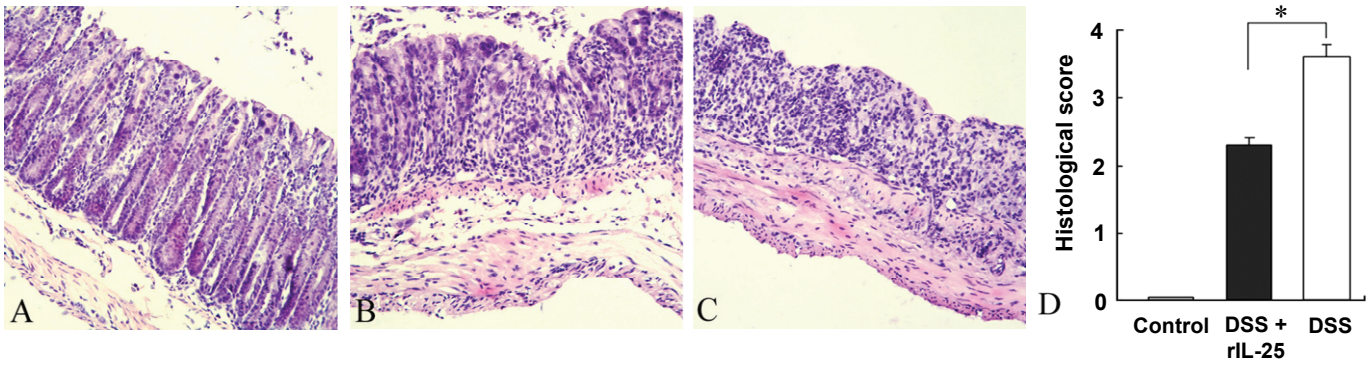

Figure 2. Histology of the distal colon of DSS-induced colitis stained with hematoxylin and eosin (×200). (A) In the control mice, reveal normal colon morphology with well defined crypts structure. (B) In the DSS + rIL-25 treated mice, reveal a grade 2 lesion, basal crypts structures and surface epithelium still remain intact with no appreciated inflammatory infiltrate. (C) In the DSS treated mice, reveal a grade 4 lesion, entered crypts structure totally destroyed, loss of surface epithelium cells with less marked epithelium erosion and moderate presence of inflammatory infiltrate. (D) Histological scores in the control, DSS + IL-25 and DSS treated mice. The data present mean ± SD (n = 6 mice /group). *$p < 0.05$.

of blood were clearly seen at day 4 while diarrhea with blood and gross bleeding were seen at day 5. On day 6, 66.6% of DSS treated mice were found to display gross bleeding. In contrast, DSS + rIL-25 treated mice displayed delayed and less severe colitis symptoms. Mice started losing body weight at day 3. However, slightly loose stools without blood were seen at day 4. On day 5, mice displayed loose stools with only minor degree of blood. On day 6, only 16.6% of mice were found to display diarrhea with gross bleeding while 83.4% of mice displayed diarrheas with blood. However, no significant difference in weight loss between DSS treated mice and DSS + rIL-25 treated mice was observed (Table 2). At autopsy, in order to assess severity of colitis, we further carefully inspected changes in colon gross appearance. As shown in Figures 1A, 1B, and 1C, in comparison with the control mice where the gross appearance of colons remain normal, the colons appearances in the DSS treated mice were found to exhibit typical signs of severe acute inflammation against a mild inflammation exhibited in the DSS + rIL-25 treated mice. Length of colons in the DSS treated mice was significantly shortened by 32% while that of the DSS + rIL-25 treated mice was shortened by 17% ($p < 0.05$) (Figure 1D). Collectively, our clinical and macroscopic observations reveal the ability of rIL-25 to delay the colitis symptoms and to reduce development and progression of inflammatory changes in colons.

Exogenous IL-25 was shown to slowdown the development and progress of acute colitis in DSS + rIL-25 treated mice

For histological colitis assessment, we further performed H&E staining of the distal colon in all groups. The DSS treated mice revealed grade 4 lesions with loss of the entire crypts, loss of the surface epithelium and the presence of inflammatory cells in the lamina propria and submucosa. In contrast, DSS + rIL-25 treated mice revealed grade 2 lesions with shortening of the basal two-thirds of the crypts, failure of the base of crypts to sit on the muscularis mucosa, intact surface epithelium and no significant inflammation. The histological score for colitis in the DSS + rIL-25 treated mice was significantly lower compared to that of the DSS treated mice ($p < 0.05$) (Figure 2). In addition to that, the survival rate of DSS + rIL-25 treated mice was significantly prolonged compared with DSS treated mice ($p < 0.05$). All of the DSS treated mice had died between days 7 and 9 while 90% of the DSS + rIL-25 treated mice died between days 8 and 11. Ten percent of the DSS + rIL-25 treated mice had survived with complete restoration of original body weight (Figure 3). These observations clearly demonstrate the ability of IL-25 to

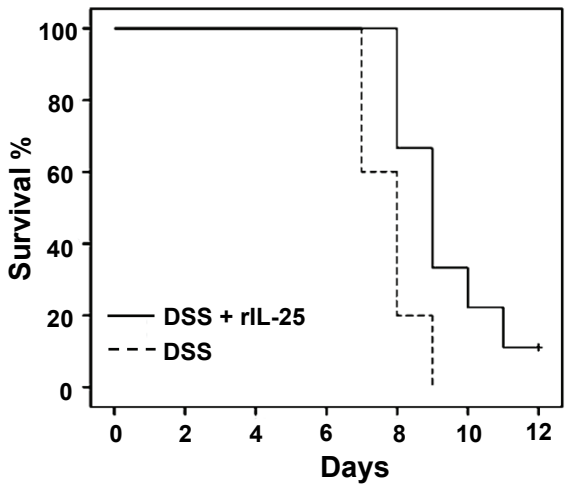

Figure 3. Kaplan-Meier survival curve of DSS + rIL-25 treated mice and DSS treated mice. Mice from DSS + rIL-25 treated group and DSS treated group were further subjected to DSS until day 7 when they were returned to normal drinking water while assessed for their survival. The DSS + rIL-25 treated mice exhibited an improved prolonged survival compared to the DSS treated mice (n = 10 mice/group), $p < 0.05$.

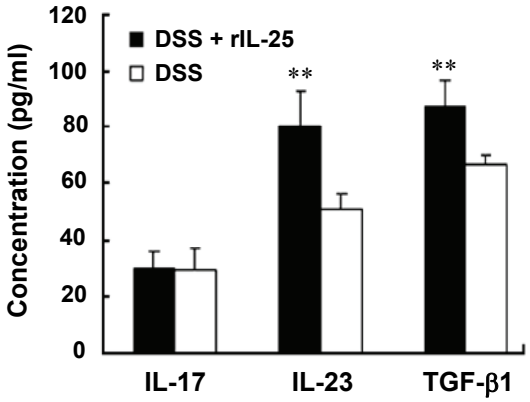

Figure 4. Levels of IL-17, IL-23 and TGF-β1 in the colonic tissues from DSS + rIL-25 treated mice and DSS treated mice. ELISA on colon tissue homogenates showed that no significant difference was observed in the production of IL-17 between DSS + rIL-25 or only DSS treated mice. The levels of IL-23 and TGF-β1 were significantly higher in the DSS + rIL-25 treated mice than those in the DSS treated mice. Data represent mean ± SD (n = 6 mice/group). $^{**}p < 0.01$.

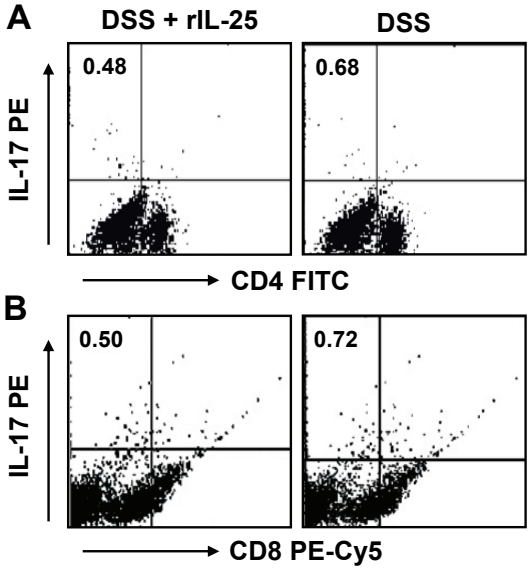

Figure 5. Flow cytometric analysis of intracellular IL-17 expression in $CD4^+/CD8^+$ T cells from DSS + rIL-25 treated mice and DSS treated mice. (A) The percentages of gated $CD4^+$ T cells expressing IL-17 in DSS + rIL-25 or only DSS treated mice. (B) The percentages of gated $CD8^+$ T cells expressing IL-17 in DSS + rIL-25 or only DSS treated mice.

resist colonic damage caused by DSS toxicity.

Significant elevation of IL-23, and TGF-β1 in the DSS + rIL-25 treated mice

To investigate the role of IL-17, IL-23, and TGF-β1 in colitis, local production of these cytokines in colon tissue homogenates was measured by ELISA. There was a significant elevation of IL-23 ($p < 0.01$) and TGF-β1 ($p < 0.01$) in the DSS + rIL-25 treated mice, but there was no significant difference found in the production of IL-17 between DSS + rIL-25 treated mice and DSS treated mice (Figure 4). Our findings indicate influential role of IL-25 in the production of IL-23 and TGF-β1 in the development of acute DSS-induced colitis.

Lower production of IL-17 by $CD4^+/CD8^+$ T cells in the development of acute DSS-induced colitis

We further performed intracellular cytokine staining to confirm the production of IL-17 by $CD4^+/CD8^+$ T cells during development of acute colitis. As judged through cytometric fluorescent intensity of intracellular staining (Figure 5), expression of IL-17 by $CD4^+/CD8^+$ T cells was found to be lower in both DSS + rIL-25 treated mice and DSS treated mice. Our findings speculated the role for $CD4^+/CD8^+$ T cells in the production of IL-17 in acute DSS-induced colitis.

Discussion

Following DSS exposure for 5 days, the DSS treated mice displayed clinical symptoms consistent with previous reports (13, 14). However, in the DSS + rIL-25 treated mice our findings clearly demonstrated that, the treatment of DSS-colitis mice with rIL-25 resulted in delayed progress of colitis, notably in terms of development of DAI, degree of histological changes and severity of inflammation. Shortening of colon, extensive edema and the presence of a considerable degree of blood in the colons of the DSS treated mice reflect a higher degree of severity of colitis in the DSS treated mice than in the DSS + rIL-25 treated mice. IL-25 is a potent inductor for the production of IL-4, IL-5, and IL-13 (8). These Th2 cytokines, particularly IL-4 and IL-13, previously have been reported to cause a number of changes in intestinal epithelial functions, such as balance of ion, mucosal fluids absorption (15), and increase in intestinal smooth muscle contraction (16). IL-13 was found primarily to mediate massive histological changes in the gastro-intestinal mucosa of mice treated with rIL-25 (8). Furthermore, activation of NF-κB has been reported in both human and murine models of IBD (17). The inhibition of NF-κB was shown to enhance intestinal inflammation as evident in NF-κB deficient mice (18, 19). IL-25 has been shown to activate NF-κB (10), suggesting that IL-25-induced NF-κB activation may be a crucial factor in attenuating colitis. Thus, these previous reports are in support of our findings in explaining the chance for rIL-25 to delay and reduce development and progression of colitis clinical symptoms through multiple channels.

Our histological findings were consistent with our clinical and macroscopic findings with remarkable morphological changes in DSS treated mice compared to DSS + rIL-25 treated mice. The histological score of colitis in the DSS + rIL-25 treated mice was significantly lower

compared to that of the DSS treated mice. In general, colitis was apparently attenuated by the administration of rIL-25 as evident in our survival study where DSS + rIL-25 treated mice exhibited an improved prolonged survival. Following an earlier report on the broad effect of IL-25 on gastrointestinal tract (8), Fallon et al. and Owyang et al. in their studies, clearly demonstrated that IL-25 can play important roles in the promotion of host defense and inflammation control (11, 12). While an anti-inflammatory role for IL-25 was shown to be due to its ability in suppressing Th1 and Th17 responses (12), the promotion of host defense was shown to be *via* inhibition of expression of type 1 cytokines (11). Yet, in a recent study, IL-25 was also reported to inhibit development and progress of inflammation in the brain and spinal cord (20). In all the studies presented above, IL-25 was clearly indicated to mediate its effects through induction of different set of cytokine genes including IL-4, IL-5 and IL-13 which are taken as the bases of its multiple effects. Therefore, our findings correlate with previous works using rIL-25, which reported an anti-inflammatory function of IL-25.

In our present study with the DSS + rIL-25 treated mice, we found an elevation of TGF-β1 and IL-23. Our results are in line with that of TNBS-induced colitis whereby both IL-25 and IL-23 were shown to appear together (21). In this most recent study, IL-25 was reported to facilitate the production of TGF-β1 through induction of IL-13 (21). TGF-β1 has long been recognized to exert an anti-inflammatory effect and under certain conditions potentates inflammation (22). Several families of growth factors have been reported to be involved in mucosal repair, restoration, modeling and resolution of inflammation after tissue damage (23). Separate studies on TNBS-induced colitis demonstrated the ability of TGF-β1 primarily to mediate counter-regulatory Th1-type mucosal inflammation and to prevent the development of colitis (24, 25). On the basis of these reports, we also suggest the role for IL-25 in suppressing mucosal damage through mediation of IL-13 and TGF-β1. However, further studies will be necessary to determine the actual role for TGF-β1 and other TGF-β1 related cytokines like IL-10 in the course of regulation of gastrointestinal tract inflammation by IL-25. Even though a number of recent studies reported a critical role for IL-23 in the pathogenesis of intestinal inflammation (26, 27), the actual causes of elevation of IL-23 cytokine in these DSS-induced colitis mice treated with rIL-25 remain uncertain. However, TGF-β1 has been shown to have the ability to up-regulate IL-23R expression to induce IL-23 responsiveness (28). Therefore, while we are trying to link an observed elevation of IL-23 in the DSS + rIL-25 treated mice with TGF-β1, an association between IL-25 and IL-23 still remain to be addressed. Moreover, we also detected the production of IL-17 in the DSS + rIL-25 treated mice and DSS treated mice but without any significant difference. In spite of the fact that the role for IL-17 in IBD is unclear, IL-17 has been shown to protect mucosal barrier functions through induction of tight junction formation (29). The role for IL-17 was lately proven through neutralization of IL-17 that resulted in exacerbation of DSS-induced colitis (30). In the present study, IL-25 was not shown to play any potential role against the production of IL-17 as it was shown in chronic inflammatory studies (12, 20). In that respect, our findings hinted at the possible influence of IL-25 in the induction of IL-23 and TGF-β1 production but not suppression of IL-17 in the development of acute DSS-induced colitis.

On the other hand, IL-23 (31, 32) and TGF-β1 (28, 33) have been strongly reported to be involved in the development of IL-17-producing $CD4^+$ T cells *via* a new T cell subset Th17. In addressing the impact of IL-23 and TGF-β1 in the differentiation of naïve T cells into Th17 cells, we further investigated the development of IL-17-producing $CD4^+/CD8^+$ T cells in splenic lymphocytes. Surprisingly, despite elevation in the levels of IL-23 and TGF-β1 production in the DSS + rIL-25 treated mice, a lower production of IL-17 by $CD4^+/CD8^+$ T cells was found in both DSS + rIL-25 treated mice and DSS treated mice. A recent study on *Mycobacterium tuberculosis* infection described a domination of production of IL-17 by γδT cells and other non-$CD4^+/CD8^+$ T cells (34). γδT cells have been shown to increase in the mucosa of ulcerative colitis patients (35). While γδT cells have been shown to provide mucosal protection at the early phase of intestinal inflammation (36), the production of IL-17 by $CD4^+/CD8^+$ T cells was suggested to occur at the late phase (34, 37). On that ground, we are of the notion that, it is likely that non-$CD4^+/CD8^+$ T cells were responsible for the determined IL-17 production. Thus we speculate the role for $CD4^+/CD8^+$ T cells in the production of IL-17 in the early development of acute DSS-induced colitis.

Up to this present moment, IL-17 remains the most widely studied cytokine in the family of IL-17. Otherwise, this is the first study to investigate and to report the role of IL-25 in the development of ulcerative colitis in the DSS-induced experimental animal model. Although the IL-25 protective mechanism in ulcerative colitis is yet to be known, we hypothesize the role of IL-25 in promoting immune responses that limit the progress of inflammation in the colon mucosa and induce production of IL-23 and TGF-β1 in the colon.

Acknowledgements

The present work was supported by a grant from the Natural Science Foundation of Liaoning province, China (No. 20072101). We are grateful to Mr. Guanghui Dong (Department of Health Statistic, China Medical University) for statistic analysis, and Ms. Yan Cao (Department of Immunology, College of Basic Medical Science, China Medical University) for technical assistance.

References

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002;347:417-429.

2. Carpenter HA, Talley NJ. The importance of clinicopathological correlation in the diagnosis of inflammatory conditions of the colon: histological patterns with clinical implication. Am J Gastroenterol. 2000;95:878-896.
3. Hanauer SB. Inflammatory bowel disease: epidemiology, pathogenesis, and therapeutic opportunities. Inflamm Bowel Dis. 2006;12 Suppl 1:S3-9.
4. Byryne FR, Viney JL. Mouse model of inflammatory bowel disease. Curr Opin Drug Disc Devel. 2006;9:207-217.
5. Egger B, Bajaj-Elliot M, MacDonald TT, Inglin R, Eysselein VE, Büchler MW. Characterization of acute murine dextran sodium sulphate colitis: cytokine profile and dose dependency. Digestion. 2000;62:240-248.
6. Kolls JK, Linden A. Interleukin-17 family members and inflammation. Immunity. 2004;21:467-476.
7. Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol. 2004;114:1265-1273; quiz 1274.
8. Fort MM, Cheung J, Yen D, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies *in vivo*. Immunity. 2001; 15:985-995.
9. Ikeda K, Nakajima H, Suzuki K, et al. Mast cells produce interleukin-25 upon FcεRI-mediated activation. Blood. 2003; 101:3594-3596.
10. Lee J, Ho WH, Maruoka M, et al. IL17E, a novel proinflammatory ligand for the IL-17 receptor homolog IL17Rh1. J Biol Chem. 2001;276:1660-1664.
11. Fallon PG, Ballantyne SJ, Mangan NE, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. J Exp Med. 2006;203:1105-1116.
12. Owang AM, Zaph C, Willson EH, et al. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. J Exp Med. 2006; 203:843-849.
13. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinico-pathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 1993;69:238-249.
14. Melgar S, Karlsson A, Michaelsson E. Acute colitis induced by dextran sulfate sodium progress to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G1328-1338.
15. Madden KB, Whitman L, Sullivan, et al. Role of STAT6 and mast cells in IL-4- and IL-13-induced alteration in murine intestinal epithelium cell function. J Immunol. 2002;169:4417-4422.
16. Zhao A, CMcDermott J, Urban JF Jr, et al. Dependence of IL-4, IL-13, and nematode-induced alterations in murine small intestinal smooth muscle contractility on Stat6 and enteric nerves. J Immunol. 2003;171:948-954.
17. Neurath MF, Fuss I, Schurmann G, et al. Cytokine gene transcription by NF-κB family members in patients with inflammatory bowel diseases. Ann NY Acad Sci. 1998;859: 149-159.
18. Erdman S, Fox JG, Dangler CA, Feldman D, Horwitz BH. Typhlocolitis on NF-κB-deficient mice. J Immunol. 2001;166: 1443-1447.
19. Artis D, Shapira S, Mason N, et al. Differential requirement for NF-κB family members in control of helminth infection and intestinal inflammation. J Immunol. 2002;169:4481-4487.
20. Kleinschek MA, Owyang AM, Joyce-Shaikh B, et al. IL-25 regulates Th17 in autoimmune inflammation. J Exp Med. 2007; 204:161-170.
21. Fichtner-Feigl S, Fuss IJ, Young CA, et al. Introduction of IL-13 triggers TGF-β1-dependent tissue fibrosis in 2,4,5,-trinitro-benzene sulfonic colitis. J Immunol. 2007;178:5859-5870.
22. Wahl SM. Transforming growth factor β: the good, the bad, and the ugly. J Exp Med. 1994;180:1587-1590.
23. Beck PL, Podolsky DK. Growth factors in inflammatory bowel disease. Inflamm Bowel Dis. 1999;5:44-60.
24. Fuss IJ, Boirivant M, Lacy B, Strober W. The interrelated roles of TGF-β and IL-10 in the regulation of experimental colitis. J Immunol. 2002;168:900-908.
25. Kitani A, Fuss IJ, Nakamura K, Schwartz OM, Usui T, Strober W. Treatment of experimental (Trinitrobenzene sulfonic acid) colitis by intranasal administration of transforming growth factor (TGF)-β1 plasmid: TGF-β1-mediated suppression of T helper cell type 1 response occurs by interleukin (IL)-10 induction and IL-12 receptor β2 chain downregulation. J Exp Med. 2000;192:41-52.
26. Kullberg MC, JanKovic D, Feng CJ, et al. IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. J Exp Med. 2006;203:2485-2494.
27. Hue S, Ahern P, Buonocore S, et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. J Exp Med. 2006; 203:2473-2483.
28. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO. Transforming growth factor-β induces development of the T_H17 lineage. Nature. 2006;441:231-234.
29. Kinugasa T, Sakaguchi T, Gu X, Reinecker HC. Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology. 2000;118:1001-1011.
30. Ogawa A, Andoh A, Araki Y, Bamba T, Fujiyama Y. Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice. Clin Immunol. 2004;110:55-62.
31. Murphy CA, Langrish CL, Chen Y, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med. 2003;198:1951-1957.
32. Vanden Eijnden S, Goriely S, De Wit D, Willems F, Goldman M. IL-23 up-regulates IL-10 and induces IL-17 synthesis by polyclonally activated naïve T cells in human. Eur J Immunol. 2005;35:469-475.
33. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in context of an inflammatory cytokine milieu supports *de novo* differentiation of IL-17-producing T cells. Immunity. 2006;24:179-189.
34. Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by γδT cells rather than CD4 T cells during *Mycobacterium tuberculosis* infection. J Immunol. 2006;177: 4662-4669.
35. Yeung MM, Melgar S, Baranov V, et al. Characterisation of mucosal lymphoid aggregates in ulcerative colitis: immune cell phenotype and TCR-γδ expression. Gut. 2000;47:215-227.
36. Kuhl AA, Pawloski NN, Grollich K, Loddenkemper C, Zeitz M, Hoffmann JC. Aggravation of intestinal inflammation by depletion/deficiency of γδT cells indifferent types of IBD animal models. J Leukoc Biol. 2007;81:168-175.
37. Stockinger B, Veldhoen M, Martin B. Th17 T cells: Linking innate and adoptive immunity. Semin Immunol. 2007;19:353-361.