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CLINICAL RESEARCH

Clinical significance of mucosal suppressors of cytokine signaling 3 expression in ulcerative colitis

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Abstract

AIM: To investigate the clinical significance of mucosal expression of suppressors of cytokine signaling 1 (SOCS1) and SOCS3 in human ulcerative colitis (UC).

METHODS: Biopsy specimens for histological analysis and mRNA detection were obtained endoscopically from the rectum of 62 patients with UC (36 men; age 13-76 years). The patients were classified endoscopically according to Matts' grade (grade 1 to 4). Expression of SOCS1 and SOCS3 mRNAs was quantified in samples by competitive reverse transcription-polymerase chain reaction (RT-PCR). GAPDH was used as an internal control for efficiency of RT-PCR and amount of RNA.

RESULTS: SOCS3 mRNA expression was significantly higher in inflamed mucosa of UC than in inactive mucosa. The level of expression was well correlated with the degree of both endoscopic and histologic inflammation. Interestingly, among the patients in remission, the group with relatively low expression of SOCS3 showed a higher rate of remission maintenance over a 12-mo period. In contrast, SOCS1 mRNA was expressed in both inflamed and non-inflamed colonic mucosa and was not correlated with the activity of colonic mucosa or prognosis.

CONCLUSION: These observations suggest that increased expression of mucosal SOCS3, but not of SOCS1, may play a critical role in the development of the colonic inflammation of UC.

Key words: Suppressors of cytokine signaling; Ulcerative colitis

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INTRODUCTION

Ulcerative Colitis (UC) is a chronic inflammatory bowel disease characterized by a dysregulated mucosal immune response[1]. Many cytokines are involved in the immunopathogenesis of UC, but the importance of cytokine signaling in UC is not fully understood.

The suppressors of cytokine signaling (SOCS) are a family of proteins that regulates the strength and duration of the cytokine signaling cascade. There are eight members of the SOCS protein family: the cytokine-inducible SH2 domain-containing protein (CIS) and SOCS1 through SOCS7[2-7]. Accumulated evidence shows that SOCS proteins can potently block the Jak/STAT pathway in the pathogenesis of various inflammatory diseases[8]. The functions of SOCS1 and SOCS3 have been well documented in mouse colitis models. In a dextran sulfate-induced mouse model of colitis, SOCS3 expression was increased at day 5 and remained high for 2 wk[9]. Transgenic mice expressing a dominant-negative mutant of SOCS3 showed increased phosphorylation of STAT3 and suffered a more severe colitis than did wild-type control mice[9]. In a 2, 4, 6-Trinitrobenzene sulphonic acid-induced mouse model of colitis, SOCS1 expression was induced in intestinal mucosal lymphocytes, and SOCS1 transgenic mice developed colitis spontaneously with age[10]. It has been already reported that both SOCS1 and SOCS3 are expressed at high levels in the inflamed colonic mucosa of humans with UC[9,10], but functional significance of local SOCS expression remains unclear. The present study was undertaken to investigate the relation between levels of SOCS mRNAs and degree of inflammation in the colonic mucosa of UC patients.

MATERIALS AND METHODS

Subjects

Our subjects were 62 patients with UC who underwent colonoscopy. The age of the patients ranged from 13 to

Table 1 Description of the criteria of Matts for endoscopic and histologic findings

Endoscopic Matts' grades
Grade 1---normal
Grade 2---mild granularity of the mucosa, with mild contact bleeding
Grade 3---marked granularity and edema of the mucosa, contact bleeding, and spontaneous bleeding
Grade 4---severe ulceration of mucosa with hemorrhage
Histologic Matts' grades
Grade 1---normal appearance
Grade 2---some infiltration of the mucosa or lamina propria with either round cells or polymorphs
Grade 3---much cellular infiltration of the mucosa, lamina propria, and submucosa
Grade 4---presence of crypt abscesses, with much infiltration of all layers of the mucosa
Grade 5---ulceration, erosion, or necrosis of the mucosa, with cellular infiltration of some or all of its layers

Table 2 Characteristics of UC patients

Characteristics		Endoscopic Matts' grade			
		1	2	3	4
n	62	19	15	26	2
Sex (male/female)	36/26	11/8	10/5	13/13	2/0
Mean age (in years) (range)	38.6 (13-76)	36.2 (13-61)	39.1 (18-65)	40.1 (18-76)	37.0 (25-49)
Duration (in years) (range)	6.0 (0.1-29)	4.7 (0.2-10)	8.2 (0.1-20)	5.6 (0.1-29)	5.0 (1-9)
Type (total/left hemi/rectal)	42/10/10	14/1/4	10/4/1	17/4/5	1/1/0
Tx					
Steroid	24	5	7	10	2
Azathioprine	7	3	1	2	1
Leukocytapheresis	9	3	2	3	1

76 years (mean ± SD, 38.6 ± 13.3 years). Rectal lesions were classified macroscopically with endoscopic Matts' classification (Table 1)[11]. Patient characteristics are shown in Table 2.

After endoscopic observation by colonoscopy (450ZH; Fuji Photo Optical Co., Ltd, Saitama, Japan, and/or 240Q; Olympus Co., Ltd, Tokyo, Japan), biopsy specimens were obtained endoscopically from these patients for histologic analysis and reverse transcription-polymerase chain reaction (RT-PCR) assay. The biopsy specimens were fixed routinely in 10% buffered formalin, stained with hematoxylin and eosin, and diagnosed histologically according to histologic Matts' classification (Table 1)[11].

Oligonucleotides

For the amplification of SOCS1, a pair of PCR primers was synthesized. The sequences were 5'-CCTTCCCCTTCCAGATTTGA-3' for the 5' primer and 5'-TCCTGGCTCCAGATACAGTT-3' for the 3' primer. For the amplification of SOCS3, the sequences of the primers were 5'-TCACCCACAGCAAGTTTCCCGC-3' for the 5' primer and 5'-GTTGACGGTCTTCCGACAGAGATGC-3' for the 3' primer. For the amplification of GAPDH, the sequences were 5'-AACATCATCCCTGCCTCTAC-3' for the 5' primer and 5'-TGGCAGGTTTTTCTAGACGG-3' for the 3' primer.

Semi-quantitative RT-PCR

Total RNA from biopsy specimens was isolated with an Rneasy Mini kit (Qiagen, Valencia, CA, USA). First-strand cDNA was synthesized from 1 μg of total RNA with random 9-mers in a 20-μL total reaction volume. Before cDNA symthesis, the RNA sample was treated with Rnase-Free Dnase (Qiagen) to eliminate possible false positives due to residual genomic DNA. PCR was performed in triplicate in 10 μL reactions. GAPDH was used as an internal control for efficiency of RT and amount of RNA. Amplification conditions consisted of denaturation for 4 min at 94℃, annealing for 1 min at 65℃, and a final extension for 7 min at 72℃. The samples were amplified for 35 cycles. PCR products were run on 2% agarose gels containing 1 × TAE. After electrophoresis, RT-PCR products of SOCS-1 (277 bp), SOCS-3 (590 bp), and GAPDH (148 bp) were analyzed and quantified with a UV Transilluminator (Toyobo, Tokyo, Japan) and NIH image software. Each sample was investigated in triplicate.

Statistical analysis

Data were analyzed with StatView software (Japanese version, Hulinks, Tokyo, Japan) on a Macintosh Computer (Apple Computer, Cupertino, CA). Groups were compared with Student's *t*-test. Differences were considered statistically significant at $P < 0.05$. Time-to-relapse curves were derived with the Kaplan-Meier method, and statistical significance was determined with the log-rank test.

RESULTS

Expression of SOCS3 mRNA in the colonic mucosa in patients with UC

SOCS3 mRNA was detected in the colonic mucosa of patients with UC (Figure 1A), consistent with previous reports[12]. Upper level shows 4 cases of endoscopic Matts 1, and lower level shows 4 cases of Matts 3 and 4. Cases 1 to 4 with Matts 1 had low expression of SOCS3, and cases 5 to 8 with Matts 3 and 4 had high levels of SOCS3, as compared to expression of GAPDH. Quantitative analysis revealed that expression of SOCS3 mRNA was significantly higher in inflamed mucosa of UC than in inactive mucosa (Figure 2A). SOCS3 mRNA levels was also significantly correlated with histological Matts' grade (Figure 2B).

Expression of SOCS1 mRNA in colonic mucosa in patients with UC

SOCS1 mRNA was expressed in both inflamed and uninflamed UC mucosa (Figure 1B); however, the level did not differ significantly (Figure 3A). The SOCS1 mRNA expression was all equally expressed compared to GAPDH. Quantitative analysis showed that there was no correlation between SOCS1 mRNA expression in the colonic mucosa and histologic Matts' grade (Figure 3B).

Relation between SOCS1 and SOCS3 mRNA expression in patients with UC

Although SOCS1 and SOCS3 are regulated by many cytokines, the relation between SOCS1 and SOCS3 remains un-

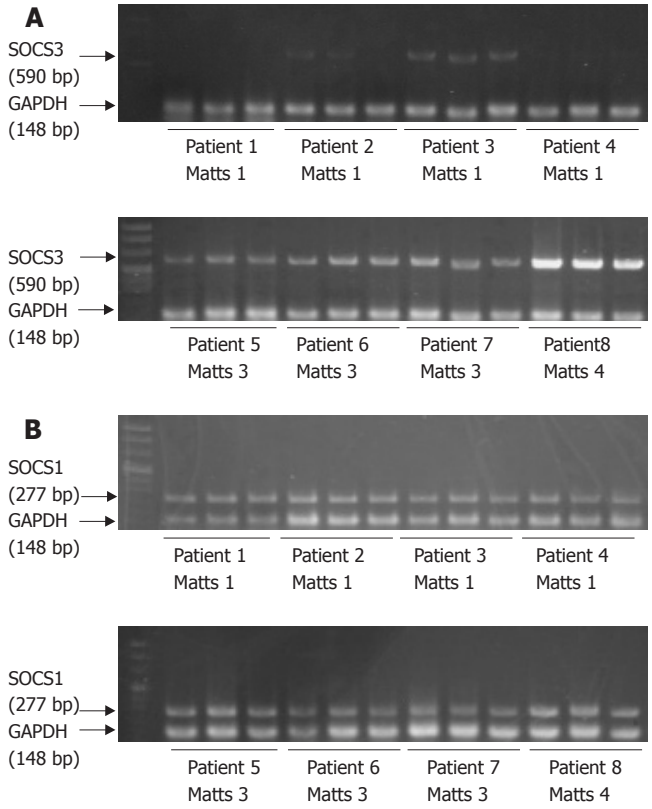

Figure 1 Expression of SOCS3 (**A**) and SOCS1 (**B**) mRNA in the colonic mucosa with UC. PCR was performed in triplicate for each sample. GAPDH was included as an internal control for efficiency of RT and amount of RNA. The colonic mucosae in cases 1 to 4 were shown endoscopically to be in remission and those in cases 5 to 8 were shown endoscopically to be active.

clear. Therefore, we evaluated the relation between expression of SOCS3 and SOCS1 mRNA and found that there was no correlation between SOCS1 and SOCS3 mRNA expression in each colonic biopsy specimen (Figure 4).

Relation between expression of SOCS mRNAs and steroid use

Because treatment with steroids is known to influence several immunological responses, we investigated the effect of steroid use on SOCS expression. There was a tendency for expression of both SOCS1 and SOCS3 to decrease in response to steroid treatment, but this difference was not statistically significant (Figure 5).

Relation between clinical activity of UC and the level of SOCS

We examined the relation between the clinical activity and expression of SOCS mRNAs. The clinical activity of UC was evaluated according to the clinical activity index (CAI), which is calculated as the sum of each parameter[13]. We found that SOCS3 mRNA expression correlated well with CAI, whereas there was no correlation between SOCS1 mRNA and CAI (Figure 6).

Expression of SOCS mRNAs in predicting relapse of UC

To determine if evaluation of SOCS expression is useful for prediction of prognosis for UC, we checked SOCS mRNA levels and CAI over a 12-mo period. Eighteen patients with UC in remission (CAI = 1 or 2) were analyzed

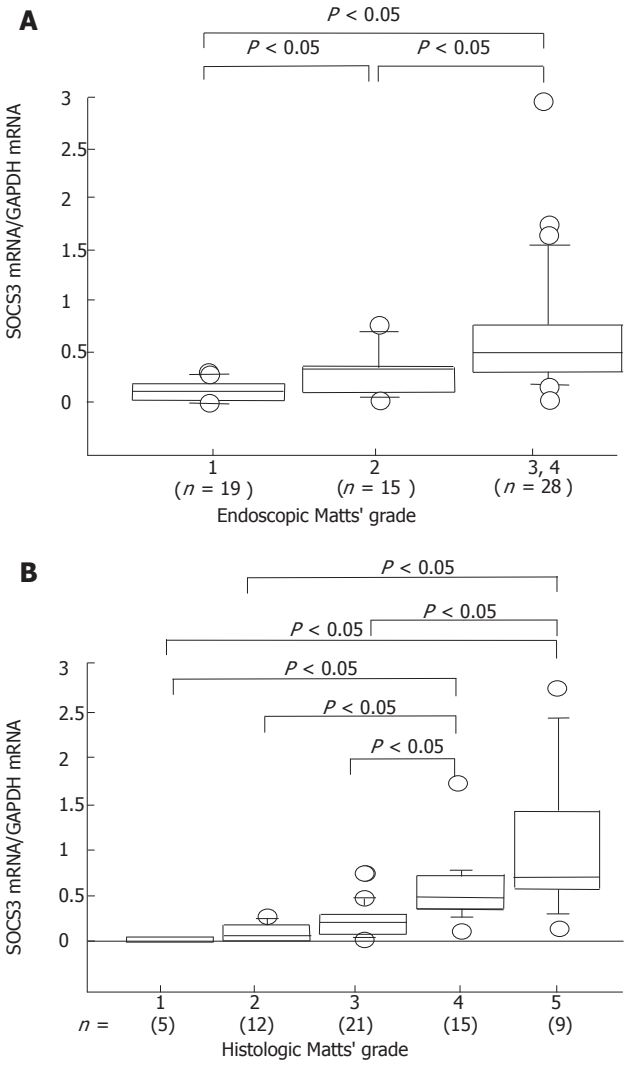

Figure 2 Relation between the degree of endoscopic (**A**) and histologic (**B**) inflammation and levels of SOCS3. The levels of SOCS3 mRNA were determined by quantitative RT-PCR. Box graphic top, bottom, and middle correspond to 75th, 25th, and 50th percentiles (median), respectively. Bar shows 5th and 95th percentiles. The mean + SD of endoscopic Matts' grade was 0.116 + 0.094 for Matts'1, 0.280 + 0.219 for Matts'2, and 0.673 + 0.599 for Matts'3 and 4. The mean + SD of histologic Matts' grade was 0.038 + 0.048 for Matts'1, 0.124 + 0.102 for Matts'2, 0.250 + 0.174 for Matts'3, 0.597 + 0.371 for Matts'4, and 1.039 + 0.821 for Matts'5. There was a significant correlation between SOCS3 expression in the colonic mucosa and Matts' grade.

for rectal SOCS expression and followed up. The patients were assessed when relapse occurred during the 12-mo period. A relapse was defined as CAI > 2. No change in treatment was made during the study. These patients were classified into two groups, low and high, according to SOCS expression levels. High SOCS3 was a SOCS3-to-GAPDH ratio of > 0.3, whereas low SOCS3 was a SOCS3-to-GAPDH ratio of ≤ 0.3. High SOCS1 was a SOCS1-to-GAPDH ratio of > 0.6, and low SOCS1 was a SOCS1-to-GAPDH ratio of ≤ 0.6. Patients who had low levels of SOCS3 (n = 14) remained in remission for the12-mo period, whereas patients with high levels of SOCS3 (n = 4) had low rate of maintaining an existing remission over the 12-mo period (Figure 7). There was no correlation between SOCS1 level and remission rate. No differences were found between the high and low SOCS3 groups in

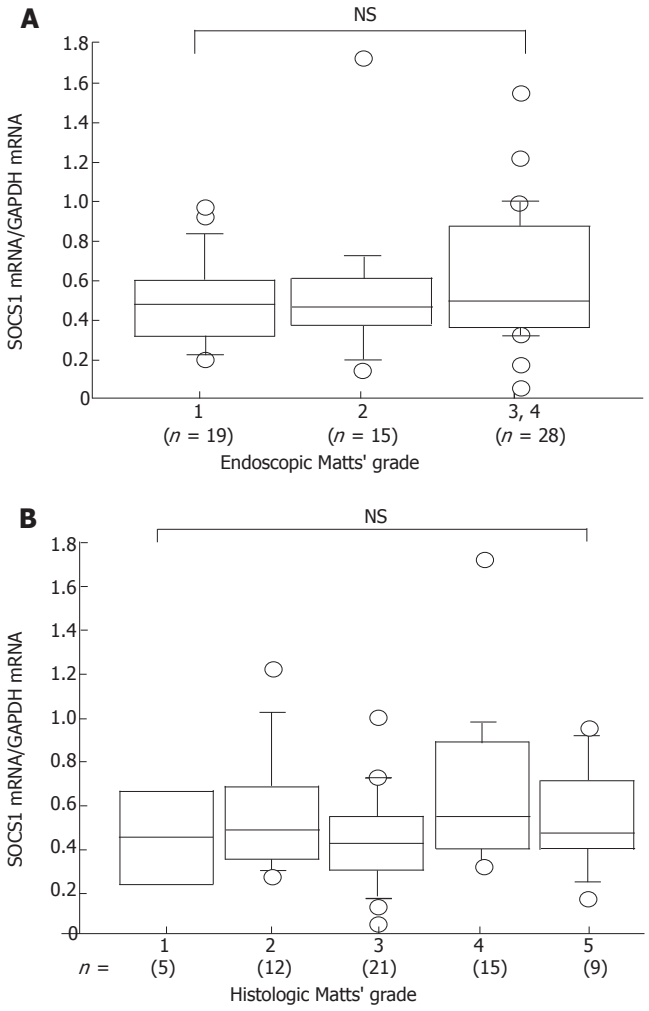

Figure 3 Relation between the degree of endoscopic (**A**) and histologic (**B**) inflammation and expression of SOCS1. Levels of SOCS1 mRNA were determined by quantitative RT-PCR. There was no correlation between the degree of inflammation and mucosal SOCS1 mRNA level.

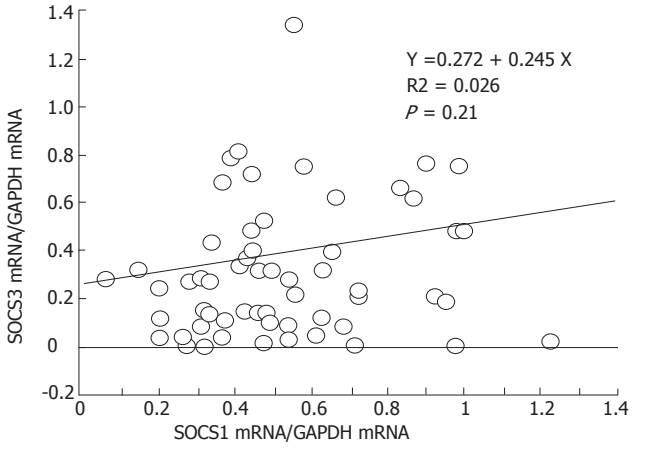

Figure 4 Relation between expression of SOCS1 and SOCS3 mRNAs. SOCS1 mRNA expression was not correlated with SOCS3 mRNA expression in colonic biopsy specimens.

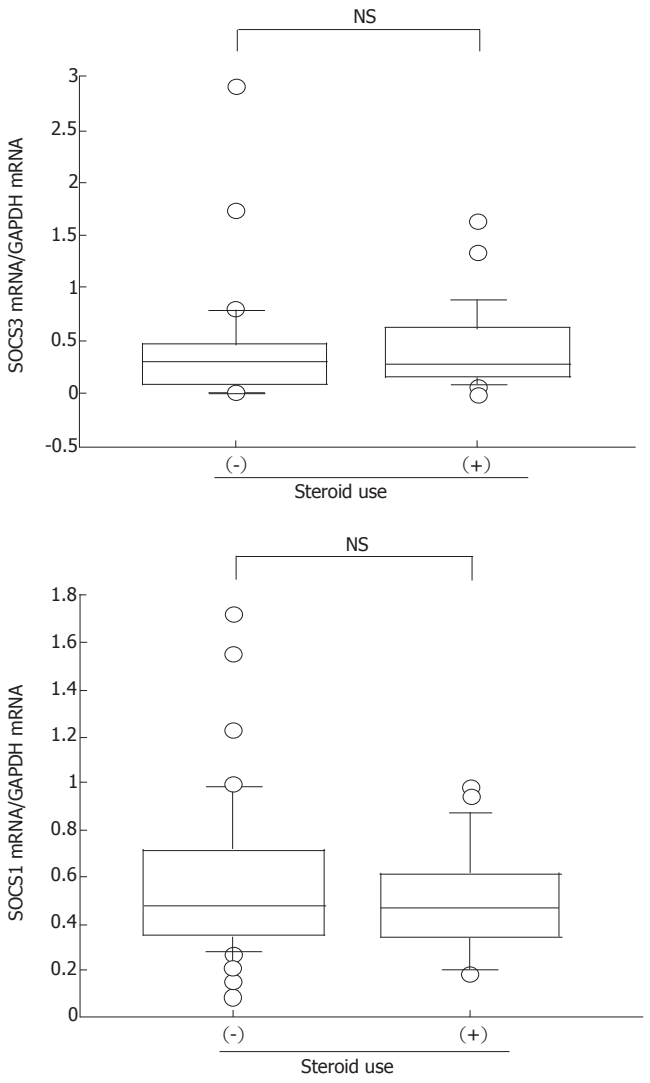

Figure 5 Effect of steroid treatment on expression of SOCS mRNAs. SOCS1 and SOCS3 levels tended to decrease in response to steroid treatment. NS: not significant.

histologic score, concomitant medications, length of remission prior to inclusion in study, or history of frequency of relapses for the individual patients. These data suggest that mucosal SOCS3 expression may be a useful prognostic marker for the maintenance of remission in UC.

DISCUSSION

In the present study, we found that expression of SOCS3 mRNA is increased according to the degree of mucosal inflammation in UC and that the level of SOCS3 may be a useful prognostic marker for patients with UC. These findings suggest that SOCS3 has a significant role in UC.

In this study, we found a close correlation between SOCS3 expression and the severity of both macroscopic and histologic inflammation of UC. In contrast, SOCS1 expression did not correlate with the severity of colonic inflammation. Expression of both SOCS1 and SOCS3 is induced by a wide variety of inflammatory and anti-inflammatory cytokines, including IL-6, IL-12, IFN-γ, and IL-10[8]. We also found that there was no correlation between the levels of SOCS1 and SOCS3. Thus, high expression of SOCS3 may not be a secondary effect of mucosal cytokine induction due to inflammatory responses.

The pathogenesis of UC is still unknown, but there

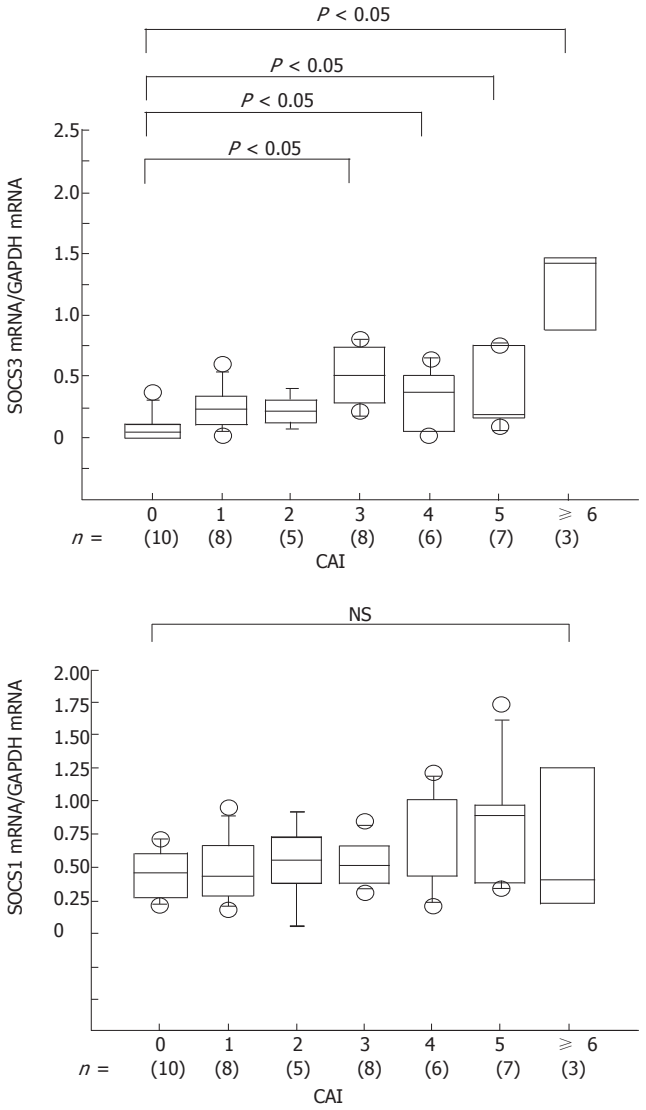

Figure 6 Relation between expression of SOCS mRNAs and CAI. SOCS3 expression is well correlated with CAI. There was no correlation between SOCS1 expression and CAI. NS: not significant.

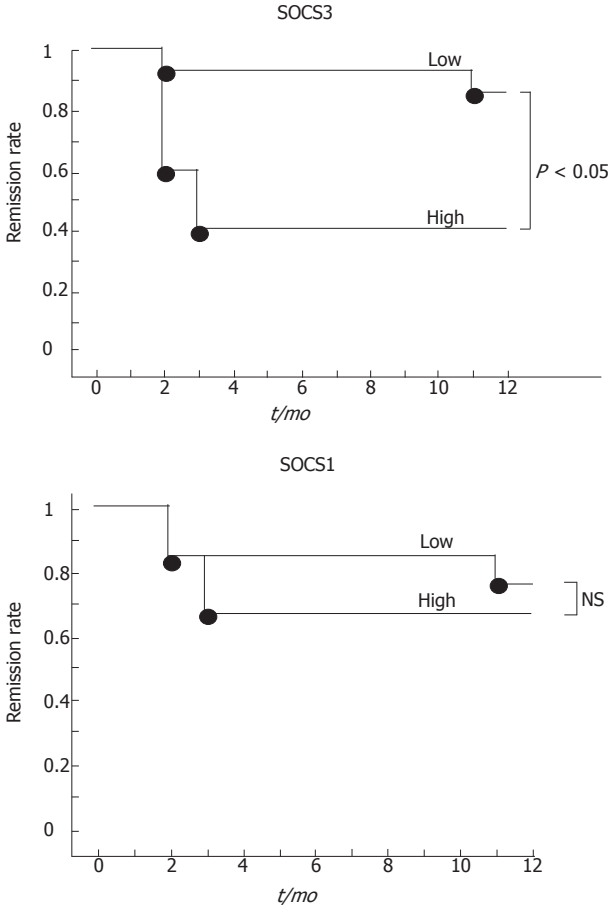

Figure 7 Kaplan-Meier time to relapse curves for patients with UC in relation to the expression of SOCS mRNAs. There is a significant difference ($P < 0.05$, log rank) in the proportion of patients who relapsed over a 12-mo period with respect to the levels of mucosal SOCS3 (> or < 0.3) at time of inclusion in the study. There was no correlation between SOCS1 and remission rate. NS: not significant.

is accumulating evidence that T-helper 2 (T_H2)-skewing immune dysregulation may be crucial in UC. Fuss *et al*[14] reported that lamina propria lymphocytes from UC secrete large amounts of IL-5 compared to those from healthy controls and Crohn's disease patients. Recently, it was reported that lamina propria mononuclear cells from patients with UC produce large amounts of IL-13, much more than those from control subjects or patients with Crohn's disease[15]. Moreover, it was also reported that IL-13 is the key effector T_H2 cytokine in UC that affects epithelial tight junctions, apoptosis, and cell restitution[16]. Taken together, these data all suggest that the T_H2-type immune response plays a crucial role in the pathogenesis of UC.

SOCS3 is expressed predominantly by T_H2 cells[17]. SOCS3 negatively regulates the IL-12 to STAT4 Th1 pathways and is possibly required for mediating T_H2 responses[18]. Interestingly, Seki *et al*[19] described a strong correlation between SOCS3 expression and the pathology of asthma and atopic dermatitis, well-known T_H2-type diseases, as well as serum IgE levels in allergic human patients. They also showed that SOCS3 transgenic mice have increased T_H2 responses in an airway hyperresponsibility model system[19]. Thus, SOCS3 has an important role in regulating the onset and maintenance of T_H2-mediated immune diseases. Recently, it was reported that overexpression of SOCS3 in lung through adenovirus SOCS3 gene transfer enhances IgG immune complex-induced lung injury[20]. SOCS3 expressed at high levels in inflamed mucosa may therefore have a pathologic role in UC.

We have not examined the cellular localization of mucosal SOCS3 expression. In mice, it has been confirmed that SOCS3 mRNA is expressed mainly in hyperplastic epithelial cells and lamina propria mononuclear cells in DSS-treated inflamed colon[9]. Han *et al*[21] confirmed that SOCS3 protein is increased and localized primarily in lamina propria lymphocytes with a lower level of expression in crypt epithelial cells in a mouse model of colitis. According to our data, expression of SOCS3 mRNA is well correlated with the degree of histologic inflammation. Therefore, accumulation of inflammatory lamina propria immune cells may be one of the main sources of increased SOCS3 mRNA. The correlation between SOCS3 expression and the severity of UC raises the possibility that high SOCS3 expression in patients may be attributable to the accumulation of T_H2 cells in the lamina propria, resulting in exacerbation of mucosal

inflammation. Further studies of the cellular localization of SOCS3 are needed.

To explore the impact of high expression of SOCS3, we examined the relation between the period of remission and SOCS3 expression. The rate of remission maintenance was significantly higher in the low SOCS3 group than in the high SOCS3 group for 1 year. This finding also suggests that mucosal SOCS3 expression is not merely the result of inflammatory change. SOCS3 may be involved in the progression of the inflammation in UC.

Treatment with steroids is known to affect cellular immune responses. We found that the patients treated with steroids showed lower levels of both SOCS1 and SOCS3 than did untreated patients. It has been reported that rat SOCS3 gene in hepatocytes is down-regulated by glucocorticoids[22]. In contrast, removal of adrenal steroids by adrenalectomy reduces SOCS3 mRNA and protein levels[23]. It has been reported that SOCS1 is involved in the response of leukemia cells to glucocorticoids[24]. These raise the possibility that steroids may directly regulate SOCS expression. Targeting SOCS3 may provide a novel strategy to UC.

In summary, our present observations suggest that mucosal SOCS3 expression may play a critical role in the development of colonic inflammation associated with UC. Monitoring of rectal SOCS3 expression may be a useful means to evaluate prognosis of patients with UC.

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