Chronic rhinosinusitis with polyps and without polyps is associated with increased expression of suppressors of cytokine signaling 1 and 3

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Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) or without nasal polyps (CRSsNP) is associated with expression of various cytokines. Suppressors of cytokine signaling (SOCS) regulate cytokine activity in a variety of cells, modulating inflammatory responses.
Objective: We analyzed the expression and distribution pattern of SOCS1 and SOCS3 in CRSwNP and CRSsNP, and their cytokine-driven expression regulation in sinus mucosa. In addition, the expression levels of various cytokines were evaluated in CRSwNP and CRSsNP.
Methods: The expression levels of SOCS1 and SOCS3 in CRSwNP and CRSsNP and in control samples were assessed by using real-time PCR, Western blot, and immunohistochemistry. Nasal epithelial cell culture was used to elucidate the effect of IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β1 on SOCS1 and SOCS3 expression in sinus mucosa. The expression levels of these cytokines were also evaluated in normal and inflammatory sinus mucosa by using real-time PCR and Western blot.
Results: The expression levels of SOCS1 and SOCS3 were increased in CRS, irrespective of the presence of nasal polyp, and they were distributed in superficial epithelium, submucosal glands, and vascular endothelium in sinus mucosa. SOCS1 was induced by IL-4, IL-13, IFN-γ, and TNF-α, while SOCS3 expression was upregulated by IL-6, IL-13, IFN-γ, and TNF-α. IL-4 and IL-13 levels were increased in CRSwNP, while IL-4, IFN-γ, TNF-α, and TGF-β1 levels were increased in CRSsNP.
Conclusion: SOCS1 and SOCS3 are increased in CRS, irrespective of nasal polyp presence. This may be a response to elevated levels of various cytokines increasingly expressed in inflammatory sinus mucosa. (J Allergy Clin Immunol 2013;■■■:■■■-■■■.)

Key words: *SOCS1, SOCS3, chronic rhinosinusitis with nasal polyps, chronic rhinosinusitis without nasal polyps, cytokines*

Abbreviations used
CRS: Chronic rhinosinusitis
CRSsNP: CRS without nasal polyps
CRSwNP: CRS with nasal polyps
CT: Computed tomography
SOCS: Suppressor of cytokine signaling

Chronic rhinosinusitis (CRS) is an inflammatory disease defined as a paranasal sinus infection that has persisted longer than 3 months. The etiology and pathophysiology of CRS are very heterogeneous and have been intensively investigated. Although they remain debatable, bacteria, virus, and fungi have been theorized to play a role as key infectious agents inciting intense host inflammatory responses.[1-4] Abnormal host responses to these triggers, including cytokine and chemokine signaling of nasal mucosa, rather than the trigger itself, have been suggested to be ultimately responsible for the persistent inflammatory process of CRS.[5,6] CRS is classified into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) on the basis of cytokine, mediator, and cellular profiles, and these 2 forms are increasingly recognized as distinct disease entities.[7] Studies examining CRS-associated cytokines showed that mixed T_H1/T_H2 cytokine profile has emerged in CRSwNP or CRSsNP, suggesting that cytokines play an important pathogenic role in CRSwNP and CRSsNP.[1,8,9]

Sustained and/or excessive action of cytokines can lead to local or systemic pathology. In both physiologic and pathologic conditions, the functions of cytokines are strictly controlled to prevent their overaction through several mechanisms, including the negative regulation of intracellular signaling.[10] Moreover, recently accumulating evidence suggests that another family of molecules, the suppressor of cytokine signaling (SOCS) family, modulates cytokine signaling.[11,12] The SOCS family of proteins consists of SOCS1 to SOCS7 and CIS1, with SOCS1 and SOCS3 being the best-characterized members of the family.[13,14] In human studies, increased SOCS1 expression is linked to asthma susceptibility through reduction of the IFN-γ–mediated T_H1 response.[15] SOCS3 is preferentially expressed in T_H2 cells, and levels are increased in patients suffering from asthma and atopic dermatitis.[16] The documented association between increased SOCS1 expression and T_H2 polarization in asthma suggests that enhanced SOCS1 expression may result in inhibition of IFN-γ signaling, skewing toward T_H2 polarization and exacerbating asthma pathology.[17] In rheumatoid arthritis in which cytokines play a key pathogenic role, the levels of SOCS1 and SOCS3 are significantly increased in peripheral mononuclear cells, indicating that SOCS expression levels are altered in rheumatoid arthritis.[18]

Considering that SOCS is a pivotal regulator for cytokine signaling, an altered level of expression of SOCS1 and SOCS3

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may play an important role in the pathogenesis of CRSwNP and CRSsNP. However, the precise participation of SOCS proteins in CRSwNP and CRSsNP has not been sufficiently evaluated.

Therefore, the present study was undertaken

1. To determine the expression levels and distribution pattern of SOCS1 and SOCS3 in normal sinus mucosa of healthy control and inflammatory sinus mucosa of patients with CRSwNP or CRSsNP to evaluate the possible effects of SOCS1 and SOCS3 in the pathogenesis of CRS, using real-time PCR, Western blot, and immunohistochemistry.
2. To investigate the expression levels of T_H2 cytokine (IL-4, IL-5, IL-6, IL-10, IL-13), T_H1 cytokine (IFN-γ, TNF-α), and TGF-β1 in normal sinus mucosa of healthy control and inflammatory sinus mucosa of patients with CRSwNP and CRSsNP that may be direct or indirect targets of SOCS protein, using real-time PCR and Western blot.
3. To elucidate whether these cytokines may regulate the expression of SOCS1 and SOCS3 in human sinus mucosa, using cultured sinus epithelial cells.

TABLE I. Patient characteristics

	Controls	CRSwNP	CRsNP
No. of patients	25	25	25
Age (y), mean range	25. 5 (22-35)	35.3 (17-45)	36.7 (20-50)
No. of females/males	6/19	9/16	7/18
Asthma history	0	0	0
Skin test result	Negative	Negative	Negative
Smoking	3/25	4/25	3/25
No. of sinus surgeries	0	0	0
SNOT-20*	3.5 ± 1.2	29.9 ± 1.5	25.5 ± 2.5
CT grade*	0	17 ± 0.7	8.6 ± 0.7
Endoscopy scores*	0	8.6 ± 0.3	2.7 ± 0.5
Total inflammatory cells†	12.7 ± 7.8	67.5 ± 10.8	62.8 ± 9.9
Eosinophils‡	1.1 ± 0.2	3.9 ± 1.1	1.9 ± 1.7
Mononuclear cells†	18.5 ± 8.9	47.9 ± 15.1	35.7 ± 12.2
Plasma cells‡	1.3 ± 1.2	4.2 ± 0.3	2.5 ± 2.3

SNOT-20, 20-ItemSino-Nasal Outcome Test.

*The data of CRSwNP and CRSsNP are significantly higher than those of controls, with difference between CRSwNP and CRSsNP.

†The data of CRSwNP and CRSsNP are significantly higher than those of controls, without difference between CRSwNP and CRSsNP.

‡The data of CRSwNP are significantly higher than those of controls and CRSsNP, without difference between CRSsNP and controls.

METHODS

Subjects

Twenty-five patients with CRSwNP, 25 patients with CRSsNP, and 25 controls were enrolled. Control subjects were patients undergoing endoscopic reduction because of blowout fracture and did not have sinus disease. Physical examination was performed in the outpatient department, which included rigid endoscopy. Endoscopic physical findings were scored according to Lanza and Kennedy.[19] A computed tomography (CT) of the sinuses with 3-mm coronal sections was obtained, and findings on sinus coronal CT scans were graded by using the Lund-MacKay CT scoring system.[20] Patient symptoms were rated by using the Sino-Nasal Outcome Test.[21] The diagnosis of CRS was made according to the current European Position Paper on rhinosinusitis and nasal polyps.[22] The presence or absence of nasal polyps was recorded on the basis of nasal endoscopy, using the definition given in the European Position Paper on Rhinosinusitis and Nasal Polyps.[19] None of the control subjects or patients with CRSwNP and CRSsNP had a history of asthma or a positive skin prick test result to a standard panel of aeroallergens. In the present study, subjects were excluded if they had any oral or topical medication including steroid, antihistamine, or antibiotics at least 3 months before the surgery. Clinical data of patients including physical findings and the CT scoring system are summarized in Table I.

Normal ethmoid sinus mucosa was obtained from the ethmoid sinus during endoscopic reduction in patients with blowout fracture. During operation, the normal-appearing ethmoid sinus mucosa, which was not injured by the fracture, was obtained as a normal control. Inflammatory ethmoid sinus mucosa lining the interior surface of the ethmoid sinus cells was, respectively, obtained from patients undergoing endoscopic sinus surgery for CRSwNP and CRSsNP. Before obtaining the tissue specimens, the institutional review boards for human beings at our institution approved the protocols and the informed consent form, and informed written consent was obtained from all patients before collecting any samples for experimental purpose.

Tissue samples were divided into 3 portions; the first portion was frozen in liquid nitrogen and stored at −80°C for subsequent RNA isolation, and the second portion was used for protein isolation. For immunohistochemistry, the third portion was fixed overnight in a freshly prepared fixative containing 4% paraformaldehyde in PBS (pH 7.4) and was embedded in paraffin wax. A part of normal sinus mucosa was used for epithelial cell culture. Sections stained with hematoxylin & eosin were examined with a light microscope to obtain a general impression of the typical pathologic features encountered in the chronically inflamed specimens. The number of total inflammatory cells, eosinophils, mononuclear cells, and plasma cells in the subepithelial layer was counted at high power magnification (×400), and 5 high-power fields was randomly selected.

Isolation and culture of epithelial cells from normal and inflammatory sinus mucosa

Human normal and inflammatory sinus mucosa were treated with 0.5% dispase in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's nutrient F12 supplemented with penicillin G sodium (50 IU/mL) and streptomycin sulfate (50 μg/mL) overnight at 4°C. Thereafter, by curettage with a sterile nasal cytology brush and agitation, nasal epithelial cells were freed from sinus mucosa and collected into a conical tube. After centrifugation, the cells were rinsed in a sterile conical tube containing Dulbecco's modified Eagle's medium/F12 and then were repeatedly centrifuged at 300*g* for 5 min at room temperature. The cells were cultured under air-liquid interface in serum-free bronchial epithelial growth medium (Lonza Walkersville, Inc, Walkersville, Md) supplemented with bovine pituitary extract (1% v/v), 5 μg/mL of insulin, 0.5 μg/mL of hydrocortisone, 50 μg/mL of gentamycin, 50 μg/mL of amphotericin B, 0.1 ng/mL of retinoic acid, 10 μg/mL of transferrin, 6.5 μg/mL of triiodothyronine, 0.5 μg/mL of epinephrine, 0.5 ng/mL of epidermal growth factor, 100 U/mL of penicillin, and 100 μg/mL of streptomycin. Cultured cells were routinely 99% to 100% positive for cytokeratin staining at the time of harvest and were used for all *in vitro* studies at first or second passage only. When cells reached 80% confluence, they were stimulated with IL-4 (10 and 100 ng/mL), IL-5 (10 and 100 ng/mL), IL-6 (10 and 100 ng/mL), IL-10 (10 and 100 ng/mL), IL-13 (10 and 100 ng/mL), TGF-β1 (1 and 10 ng/mL), TNF-α (10 and 100 ng/mL), or IFN-γ (10 and 100 ng/mL) for 24 h. These cytokines were purchased from PeproTech (Rocky Hill, NJ). Thereafter, cultured cells after stimulation were harvested and used for real-time PCR or for Western blot to analyze the expression levels of SOCS1 and SOCS3.

Real-time RT-PCR analysis

Total RNA was extracted from frozen sinus tissues and cultured epithelial cells stimulated with cytokine using Trizol reagent (Life Technologies, Rockville, Md). Total RNA (1 μg) from each sample was reverse-transcribed in 20 μL of a reaction mixture containing 2.5 U of MML-V (RT; GIBCO BRL, Grand Island, NY) and 50 pmol of random hexanucleotides at 42°C for 60 minutes. RNA integrity and the success of the reverse transcription reaction were monitored by PCR amplification of glyceraldehyde-3-phosphate dehydrogenase transcripts. The primer sequences of each gene used in the study are listed in Table II. Real-time PCR was carried out by using an iCycler (BioRad Laboratories, Hercules, Calif). Each PCR reaction included 50 ng of cDNA, 12.5 μL of iTAQ SYBR supermix, and 200 nM of primers. PCR conditions

TABLE II. Sequences of PCR primers

Primer	Size (bp)	Sequence
SOCS1	109	S: 5′-CGCCCTTAGCGTGAAGAT-3′ AS: 5′-CTCGAAGAGGCAGTCGAAG-3′
SOCS3	83	S: 5′-TTCAGCTCCAAGAGCGAGTA-3′ AS: 5′-GTCACTGCGCTCCAGTAGAA-3′
IL-4	169	S: 5′-TTGCTGCCTCCAAGAACACAACTG-3′ AS: 5′-TTCCTGTCGAGCCGTTTCAGGAAT-3′
IL-5	83	S: 5′-TAGCTCTTGGAGCTGCCTACGTGTAT-3′ AS: 5′-AAGCAGTGCCAAGGTCTCTTTCAC-3′
IL-6	88	S: 5′-AAATTCGGTACATCCTCGACGGCA-3′ AS: 5′-AGTGCCTCTTTGCTGCTTTCACAC-3′
IL-10	109	S: 5′-TCCTTGCTGGAGGACTTTAAGGGT-3′ AS: 5′-TGTCTGGGTCTTGGTTCTCAGCTT-3′
IL-13	120	S: 5′-TGGTCAACATCACCCAGAACCAGA-3′ AS: 5′-AGCCTGACACGTTGATCAGGGATT-3′
TGF-β1	157	S: 5′-CGACTACTACGCCAAGGA-3′ AS: 5′-GAGAGCAACACGGGTTCA-3′
TNF-α	131	S: 5′-AAGCCCTGGTATGAGCCCATCTAT-3′ AS: 5′-ATGATCCCAAAGTAGACCTGCCCA-3′
IFN-γ	182	S: 5′-TGCAGGTCATTCAGATGTAGCGGA-3′ AS: 5′-TGTCTTCCTTGATGGTCTCCACACTC-3′
GAPDH	122	S: 5′-ATCATCCCTGCCTCTACTGG-3′ AS: 5′-GTCAGGTCCACCACTGACAC-3′

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

for 40 cycles were 95°C denaturation for 30 seconds, 59°C annealing for 30 seconds, and 72°C extension for 30 seconds. The mean threshold cycle values were normalized to glyceraldehyde-3-phosphate dehydrogenase, and the relative mRNA levels of target genes were analyzed with the $2^{-\Delta\Delta Ct}$. Experiments were performed in triplicate for each data point.

Immunohistochemical and Western blot analysis

Immunohistochemical staining was performed by using a peroxidase-labeled streptavidin-biotin technique. Briefly, paraffin sections (4 μm) were cut and placed on poly-L-lysine–coated glass slides. Sections were treated with 0.03% H_2O_2 in methanol for 15 min to quench endogenous peroxidase activity. After washing in PBS, sections were then incubated overnight at 4°C with a 1:250 dilution of anti-SOCS1 antibody or a 1:250 dilution of anti-SOCS3 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif). Thereafter, each section was washed with PBS, incubated with biotinylated antirabbit IgG (Vector Laboratories, Inc, Burlingame, Calif), and treated with avidin-biotin complex. The color was developed by using 3,3′-diaminobenzidine. The negative immunohistochemical control procedure included omission of the primary antibodies and replacement of the primary antibodies with normal rabbit IgG in appropriate concentrations.

For Western blot analysis, frozen sinus tissue and cultured epithelial cells harvested after stimulation were crushed and vigorously vortexed in a buffer solution containing 50 mM of Tris-HCl (pH 8.0), 150 mM of NaCl, and a proteinase inhibitor cocktail. The extracted protein (50 μg) was resuspended in SDS sample buffer and boiled for 5 minutes. Equal amounts of total protein were separated by 12% SDS-polyacrylamide gel electrophoresis and transferred to immobilon (Millipore, Bedford, Mass). The blots were blocked for 1 hour at room temperature with PBS-Tween-20 containing 1% skim milk and then incubated with a 1:1500 dilution of anti-SOCS1 or a 1:1500 dilution of anti-SOCS3 antibody or a 1:1000 dilution of anticytokine antibody (IL-4, IL-5, IL-6, IL-10, IL-13, TGF-β1, TNF-α, or IFN-γ) in PBS-T overnight at room temperature. Antibody reactions were detected by using the ECL detection kit (GE Healthcare and Amersham Biosciences, Piscataway, NJ), followed by detection of chemiluminescence on x-ray film. As an internal control, the expression of β-actin was analyzed in parallel blots by using β-actin antibody (Sigma-Aldrich, St Louis, Mo). The intensity of detected bands was quantified by using Scion Image Beta 4.0.2 (Scion Corporation, Frederick, Md). The relative intensities of each protein signal were obtained by dividing the intensities of each protein signal by the intensities of β-actin signals.

Statistical analyses

Statistical analyses were carried out by using SPSS for Windows (version 16.0.0; SPSS, Chicago, Ill). The age differences, 20-item Sino-Nasal Outcome Test, CT score, endoscopic score, and number of inflammatory cells in the 3 groups were calculated by using 1-way ANOVA, and the Bonferroni *post hoc* correction was applied. One-way ANOVA, the Bonferroni *post hoc* correction ($\alpha = 0.0167$), and Tukey test were performed to establish statistically significant differences in values obtained from the nasal mucosa of healthy controls and patients with CRSwNP and CRSsNP, including the data evaluated by real-time PCR and Western blot analysis. The Kolmogorov-Smirnov test showed that the distribution of each data was normal. The level of significance was set at P less than .05. Mann-Whitney U tests were carried out to compare differences in the expression levels of SOCS1 and SOCS3 mRNA and proteins between cytokine-stimulated and nonstimulated control groups.

RESULTS

Expression levels and distribution pattern of SOCS1 and SOCS3 in sinus mucosa

To identify factors that may alter cellular responsiveness to cytokines in CRS, we analyzed the expression of SCOS1 and SOCS3 molecules in sinus mucosa of 25 control subjects, 25 patients with CRSwNP, and 25 patients with CRSsNP. The quantitative gene expression of SOCS1 and SOCS3 was determined by using real-time PCR, and the results are displayed in Fig 1, *A*. Both SOCS1 and SOCS3 mRNA were detectable in all sinus mucosa samples, and the expression levels of both mRNA were significantly increased in patients with CRSwNP and CRSsNP than in control subjects (Fig 1, *A*). However, there was no significant difference between CRSwNP and CRSsNP. Western blot analysis also detected SOCS1 and SOCS3 protein in sinus mucosa of control subjects and patients with CRSwNP and CRSsNP (Fig 1, *A*). The relative abundance of SOCS1 and SOCS3 with respect to β-actin was significantly increased in patients with CRSwNP and CRSsNP than in control subjects but was not significantly different between patients with CRSwNP and CRSsNP (Fig 1, *B*). These results indicate that the expression levels of SOCS1 and SOCS3 were increased in inflammatory sinus mucosa of patients with CRS, irrespective of polyp presence, symptom severity, and the number of infiltrated inflammatory cells, compared with normal sinus mucosa of healthy controls (Table I).

The distribution pattern of SOCS1 and SOCS3 in sinus mucosa of control subjects and patients with CRSwNP and CRSsNP was analyzed by immunohistochemistry. Immunohistochemical staining showed a similar pattern in all samples of normal sinus mucosa of control subjects and inflammatory sinus mucosa obtained from patients with CRSwNP and CRSsNP, respectively. In normal sinus mucosa and inflammatory sinus mucosa of CRSwNP and CRSsNP, SOCS1, and SOCS3 were localized to the superficial epithelium, submucosal glands, and vascular endothelium (Fig 1, *C*). Nevertheless, the distributional pattern of SOCS1 and SOCS3 was not different among normal and inflammatory sinus mucosa of CRSwNP and CRSsNP. These findings were completely absent in the negative immunohistochemical control procedure including omission of the primary antibodies,

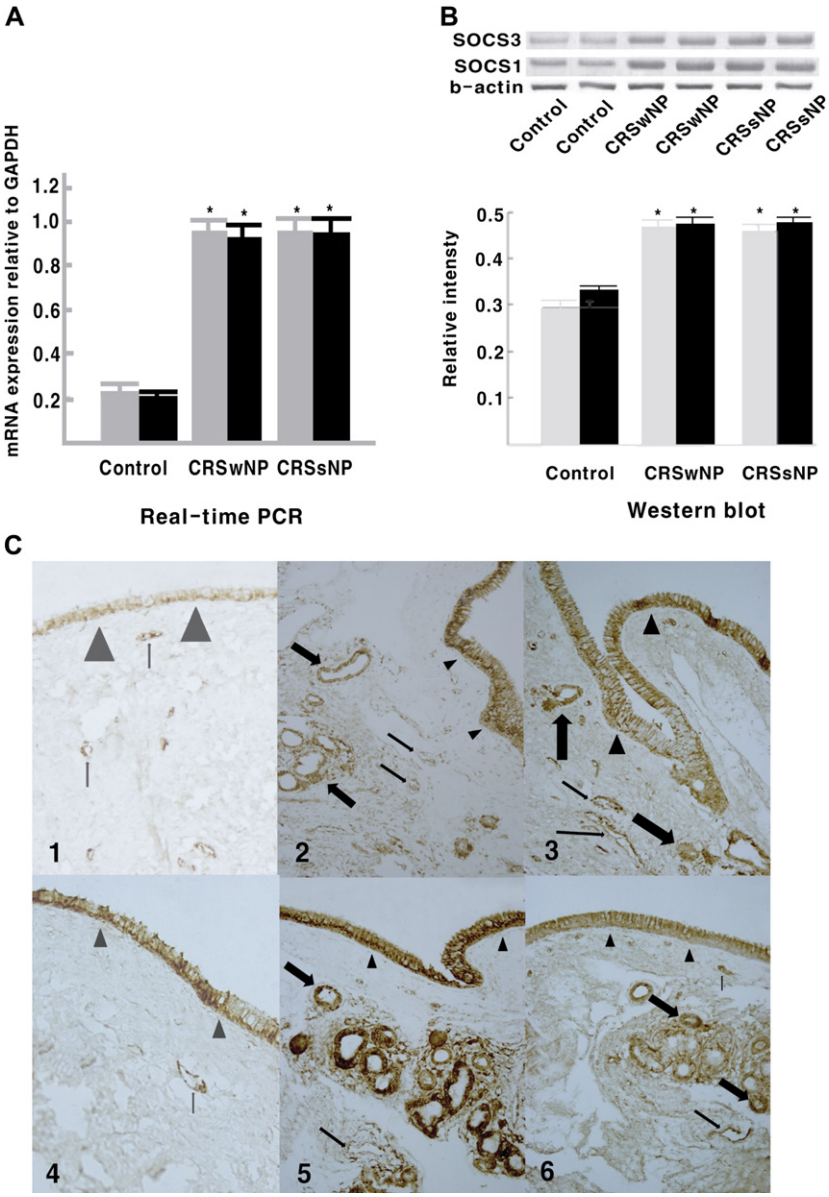


FIG 1. Real-time PCR **(A)** and Western blot analysis **(B)** of SOCS1 and SOCS3 expression in normal and inflammatory sinus mucosa of CRSwNP and CRSsNP. *Asterisk* indicates significant difference in the expression levels of SOCS1 and SOCS3 between normal control and CRSwNP or CRSsNP ($P < .05$). *White bar* denotes SOCS1, and *black bar* indicates SOCS3. Results are expressed as mean ± SD. The panels of Western blot show representative data. **C,** Immunohistochemical localization of SOCS1 (1, 2, 3) and SOCS3 (4, 5, 6) in normal (1, 4) and inflammatory sinus mucosa of CRSwNP (2, 5) and CRSsNP (3, 6). In normal and inflammatory sinus mucosa, SOCS1 and SOCS3 were detected in the surface epithelium *(arrowhead),* submucosal glands *(thick arrow),* and vascular endothelium *(thin arrow).* Original magnification ×100. *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase.

or replacement of the primary antibodies by normal rabbit IgG in appropriate concentrations (data not shown).

Expression levels of cytokines and mediators in sinus mucosa

The profile of T_H1 and T_H2 cytokines and TGF-β1 expression was compared between CRSwNP and CRSsNP, and in comparison with normal controls. mRNA and proteins for all cytokines tested in the present study were detected in all normal sinus mucosa of control subjects and inflammatory sinus mucosa of patients with CRSwNP and CRSsNP tested. The expression levels of IL-4 mRNA and proteins were increased in CRS, irrespective of nasal polyp, compared with normal controls (Fig 2, *A* and *B*). The expression levels of IL-5, IL-6, and IL-10 mRNA and proteins were not different among normal and inflammatory sinus mucosa of CRSwNP and CRSsNP. However, IL-13 mRNA and protein were increased in CRSwNP, whereas IFN-γ, TNF-α, and TGF-β1 mRNA and proteins were increased in CRSsNP (Fig 2, *A* and *B*). TGF-β1 expression levels in CRSwNP were decreased compared with normal sinus mucosa (Fig 2, *A* and *B*).

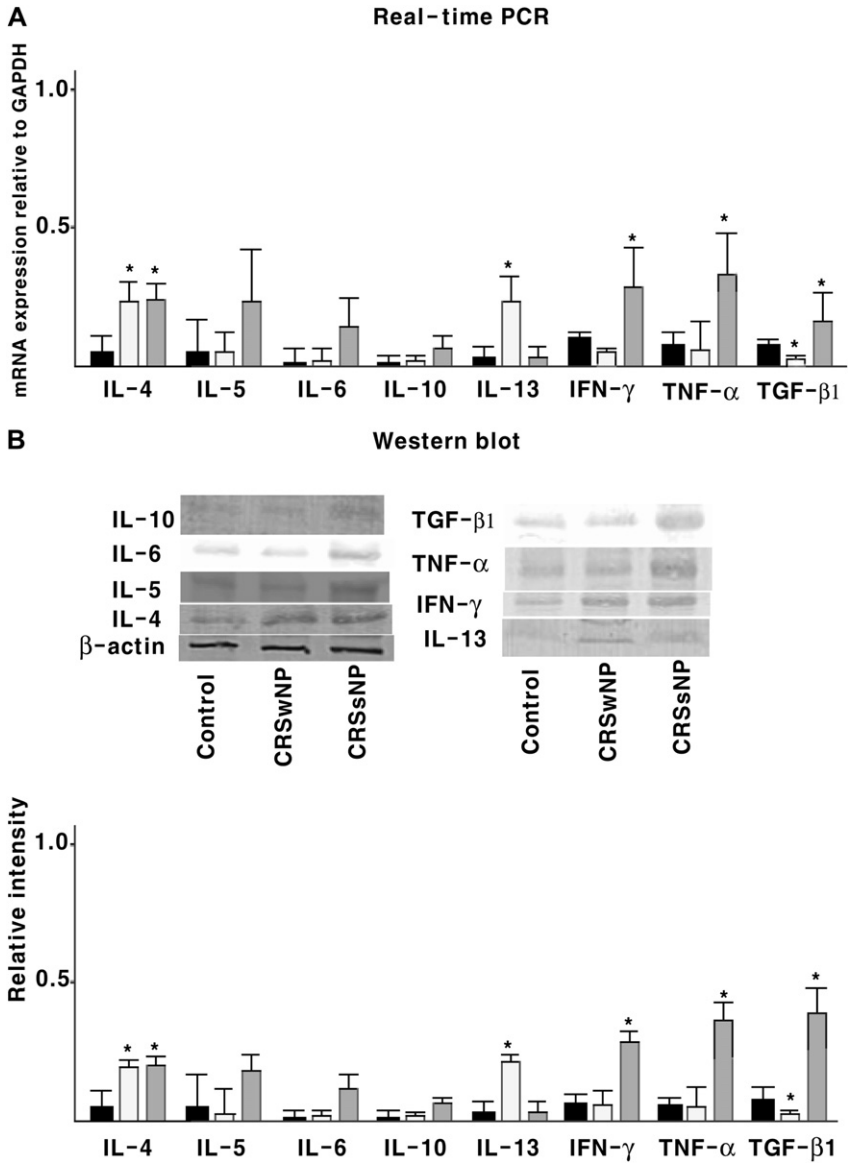


FIG 2. Real-time PCR **(A)** and Western blot analysis **(B)** of IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β1 expression levels in normal and inflammatory sinus mucosa of CRSwNP and CRSsNP. *Black bar* indicates normal sinus mucosa (n = 25), *white bar* indicates CRSwNP (n = 25), and *gray bar* represents CRSsNP (n = 25). Results are expressed as mean ± SD. The panels of Western blot show representative data. *Asterisk* indicates statistically significant difference in the expression levels of each cytokine among normal and inflammatory sinus mucosa of CRSwNP and CRSsNP ($P < .05$). *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

Effect of cytokines on the expression levels of SOCS1 and SOCS3 in sinus mucosa

Considering the current knowledge on the regulation of SOCS expression, cytokines may be involved in the upregulation of SOCS levels in sinus mucosa. To examine which of these cytokines is most potent in inducing the SOCS1 and SOCS3 expression in the epithelial cells, the cells derived from normal and inflammatory sinus mucosa were cultured in the presence of cytokines for 12 h or 24 h, and the expression levels of SOCS1 and SOCS3 mRNA and proteins were then determined. IL-4, IL-13, IFN-γ, and TNF-α resulted in the increased production of SOCS1 (Fig 3, *A-D*), while SOCS3 expression was induced by IL-6, IL-13, IFN-γ, and TNF-α (Fig 4, *A-D*). Of these cytokines, IFN-γ was the best inducer of SOCS1 and SOCS3 in epithelial cells (Figs 3 and 4).

DISCUSSION

Numerous studies have suggested that many cytokines could play important roles in the pathogenesis of CRS.[1,6,23] We hypothesized that SOCS proteins may contribute to the maintenance of organ homeostasis by preventing harmful cytokine responses in CRS. In this respect, we considered it necessary to perform a detailed analysis of SOCS expression in normal controls and patients with CRS. In this study, we show that the expression of SOCS1 and SOCS3 at the mRNA and protein levels is increased

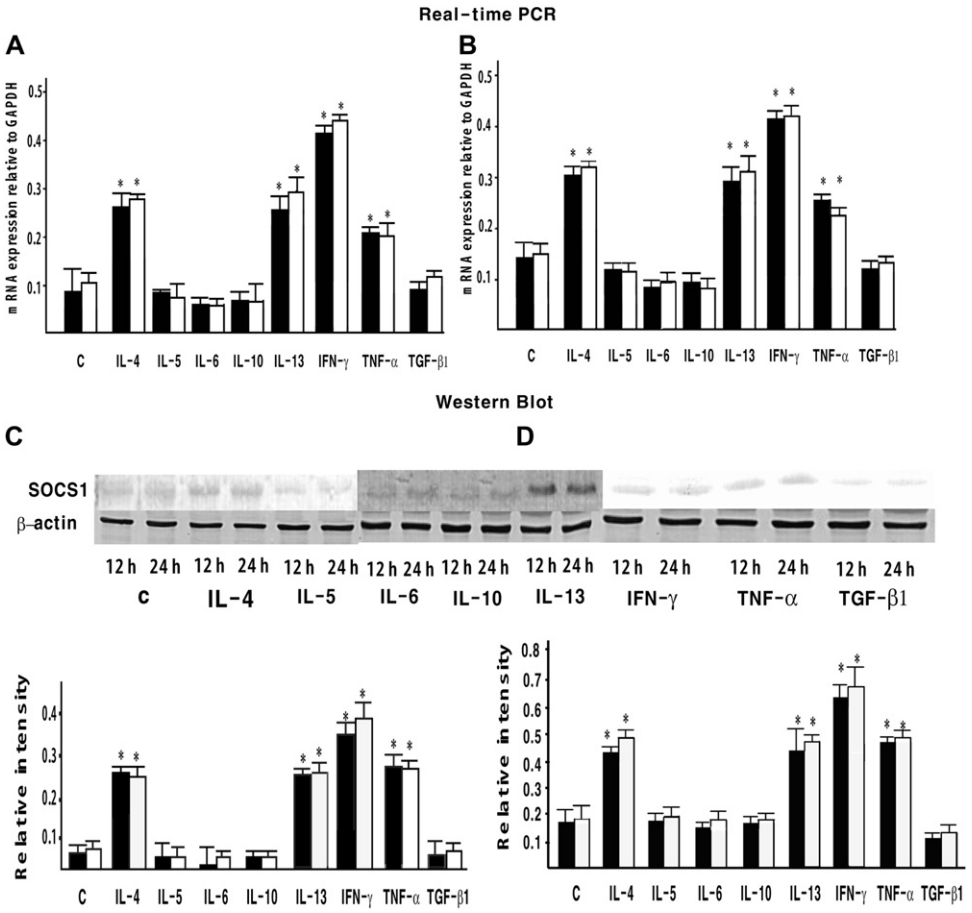


FIG 3. Real-time PCR **(A, B)** and Western blot analysis **(C, D)** of SOCS1 expression levels in cultured epithelial cells derived from normal **(A, C)** and inflammatory sinus mucosa **(B, D)** after stimulation with IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β1, respectively, for 12 hours or 24 hours. *Black bar* indicates stimulation for 12 hours (n = 6), and *white bar* indicates stimulation for 24 hours (n = 6). Results are expressed as mean ± SD. The panels of Western blot show representative data of cultured normal epithelial cells. *Asterisk* indicates statistically significant difference in SOCS1 expression levels. *GAPDH*, Glyceraldehyde-3-phosphate dehydrogenase.

in sinus mucosa of patients with CRSwNP and CRSsNP than in normal sinus mucosa of controls. In normal and inflammatory sinus mucosa, SOCS1 and SOCS3 were commonly localized to the superficial epithelium, submucosal glands, and vascular endothelium. The stimulation of cultured epithelial cells with IL-4, IL-13, IFN-γ, and TNF-α resulted in the upregulation of SOCS1 expression, while SOCS3 expression was induced by IL-6, IL-13, IFN-γ, and TNF-α. Taken together, our data indicate that SOCS1 and SOCS3 are constitutively expressed in healthy human sinus mucosa and upregulated in inflammatory sinus mucosa of patients with CRSwNP and CRSsNP and suggest that SOCS1 and SOCS3 may be induced by cytokines increasingly expressed in inflammatory sinus mucosa, playing an important role in the pathophysiology of CRS, irrespective of nasal polyp presence. This is the first detailed analysis comparing the expression levels and distribution pattern of SOCS1 and SOCS3 in inflammatory sinus mucosa of patients with CRS and in healthy sinus mucosa of controls, and provides data useful in the development of specific target treatment strategies for the management of these patients.

Evidence is emerging for the involvement of SOCS in human chronic inflammatory diseases, including asthma, allergic rhinitis, and atopic dermatitis.[24,25] Furthermore, the levels of SOCS1 and SOCS3 are significantly increased in peripheral blood mononuclear cells from patients with rheumatoid arthritis when compared with healthy volunteers, and in patients with chronic gingivitis and periodontitis.[18,26] SOCS3 is increased in the intestinal mucosa derived from patients with Crohn's disease and ulcerative colitis.[27,28] To avoid the effects of mediators relating to allergic rhinitis or asthma on the expression of SOCS1 and SOCS3 in sinus mucosa, subjects who do not have symptoms and signs for allergic rhinitis and asthma were enrolled in the present study. Therefore, consistent with the results mentioned above, our study also shows that SOCS1 and SOCS3 mRNA and proteins are increased in nonallergic or nonasthmatic inflammatory sinus mucosa of patients with CRSwNP and CRSsNP than in healthy nasal mucosa of controls. However, the question of whether the role of SOCS in CRS is beneficial or detrimental in inflammatory processes remains to be established. Other studies demonstrated that the inhibition of SOCS expression leading to sustained STAT activation contributes to the progression of chronic inflammatory diseases.[28-30] Recently, a mimetic peptide containing the kinase inhibitory region of SOCS1 was proven to effectively protect against virus infection.[31] In an

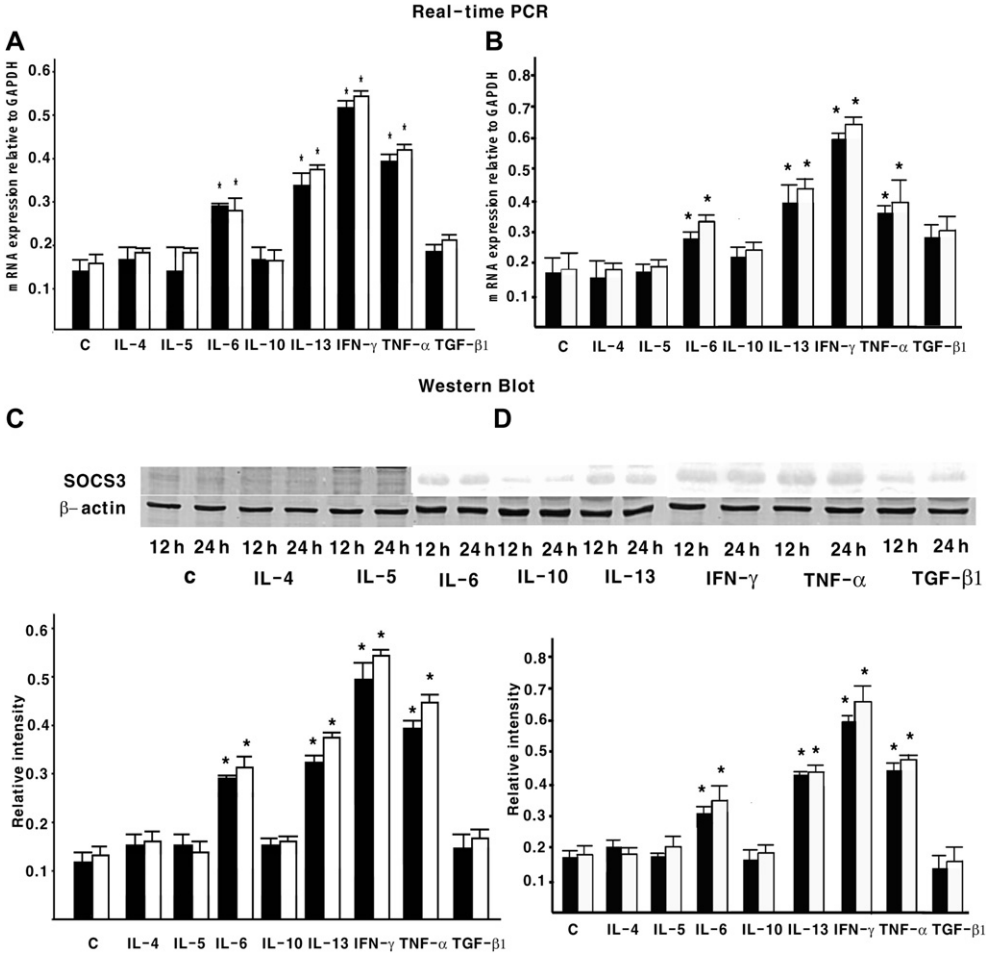


FIG 4. Real-time PCR **(A, B)** and Western blot analysis **(C, D)** of SOCS3 expression levels in cultured epithelial cells derived from normal **(A, C)** and inflammatory sinus mucosa **(B, D)** after stimulation with IL-4, IL-5, IL-6, IL-10, IL-13, IFN-γ, TNF-α, and TGF-β1, respectively, for 12 hours or 24 hours. *Black bar* indicates stimulation for 12 hours (n = 6), and *white bar* indicates stimulation for 24 hours (n = 6). Results are expressed as mean ± SD. The panels of Western blot show representative data of cultured normal epithelial cells. *Asterisk* indicates statistically significant difference in SOCS3 expression levels. *GAPDH,* Glyceraldehyde-3-phosphate dehydrogenase.

ovalbumin-induced model of allergic airway disease, allergen exposure induced upregulated expression of SOCS1, and the induction of SOCS1 attenuated allergen-induced airway responses.[32] Furthermore, SOCS1 and SOCS3 are induced by cytokines that signal through the JAK/STAT pathway, and function as endogenous inhibitors of the activation of JAK/STAT, reducing the cellular effects of cytokines and also inhibiting their expression.[11,12] On the basis of these observations, we can speculate that SOCS1 and SOCS3 are induced in CRS as a compensatory mechanism to suppress chronic inflammation, providing the negative regulation of inflammatory cytokine networks, and suggest that the modulation of SOCS expression might have therapeutic potential in CRS. However, to evaluate the possible effects of SOCS1 and SOCS3 to attenuate chronic inflammatory process in CRS, further studies using animal models of CRS are required.

The mechanism that mediates the upregulation of SOCS1 and SOCS3 in CRS remains unknown. One possible mechanism is that the elevated levels of various cytokines in *CRS* may result in cytokine-induced elevation of SOCS1 and SOCS3 as part of the normal transcriptional response, based on the fact that most SOCS proteins are induced by cytokines, and therefore act in a classical negative feedback loop to inhibit cytokine signal transduction.[10-12] In the present study, stimulation of cultured epithelial cells with IL-4, IL-13, IFN-γ, and TNF-α results in upregulated expression of SOCS1, while SOCS3 expression is induced by IL-6, IL-13, IFN-γ, and TNF-α. Therefore, these data provide evidence that these cytokines may contribute to the induced production of SOCS1 and SOCS3 in the sinus mucosa of patients with CRSwNP and CRSsNP. The regulation of SOCS1 and SOCS3 expression has been previously studied in a variety of cell types, but remains somewhat controversial. In central nervous system cells such as astrocytes, microglia, oligodendrocytes, and neurons, SOCS1 and SOCS3 expression is induced by stimuli such as IL-4, IL-6, IL-10, and IFN-γ. In T cells, SOCS1 and SOCS3 are induced by IL-4, IL-6, IL-12, and IFN-γ. In macrophages, IFN-γ induces SOCS1 and SOCS3, but stimulation with TNF-α results in the upregulation of SOCS3 expression.[33] In peripheral mononuclear cells, IFN-γ, IL-6, and IL-10 all induce SOCS1 and SOCS3 expression, with IFN-γ being the best inducer of SOCS1, while IL-10 induces highest levels of

SOCS3.[18] Another study using cultured human airway smooth muscle cells showed that IL-4, IL-13, and IFN-γ all increased SOCS-1, but not SOCS-3, mRNA expression.[34] Taken together, the dichotomous results concerning the effects of cytokines on SOCS1 and SOCS3 expression may reflect differences in tissue condition between the various studies in the literature. Additional studies to elucidate the molecular mechanisms linking SOCS1 and SOCS3 expression will be needed to further our understanding of the regulatory molecules.

Bacterial inflammation involved in the pathogenesis of CRS is partly mediated by bacterial cell membrane components lipopolysaccharide and lipoteichoic acid, which can induce SOCS expression in CRS.[35-37] Recently, adherent bacterial populations known as biofilms have been proposed as a potential environmental trigger in CRS, contributing to T_H2-polarized inflammation, irrespective of polyp status.[38] Collectively, these data suggest that increased expression of SOCS1 and SOCS3 molecules in CRS may be induced by various factors as well as cytokines relevant to the pathogenesis of CRS. These suggestions are supported by data from the literature demonstrating that in addition to cytokines, SOCS1 and SOCS3 expression is induced by many pathologic stimuli (eg, lipopolysaccharide, chemokines, and epidermal growth factor), indicating their involvement in many biologic processes.[11,12]

Studies investigating the pattern of sinus mucosal inflammation by measurement of inflammatory mediators demonstrated that CRSsNP is characterized by a T_H1 polarization with high levels of IFN-γ and TGF-β, while CRSwNP shows T_H2 polarization with high levels of IL-4, IL-5, and IL-13.[1,23,39,40] Although the current data are not completely matched to these results, enhanced SOCS1 and SOCS3 expression may result in the inhibition of IFN-γ signaling, skewing toward T_H2 polarization and at least contributing to the development of CRSwNP. These suggestions are supported by the results that T_H1 downregulation by SOCS1 and SOCS3 may result not only in an accelerated T_H2 response but also in T_H2-related diseases such as allergy.[41] SOCS1 is an essential physiologic negative regulator of IFN-γ signaling that is crucial for the induction-appropriate immune responses. SOCS3 also represses IFN-γ signaling but less than SOCS1.[41] However, it should be pointed out that although we discovered the effect of these cytokines on upregulated SOCS1 and SOCS3 expression *in vitro*, the role of SOCS1 and SOCS3 in cytokine regulation in CRS *in vivo* still need to be defined.

In conclusion, this is the first report exploring the association between SOCS and CRS. Our results suggest that SOCS1 and SOCS3 may be involved in the pathogenesis of CRSwNP and CRSsNP, and can be modulated differentially by different cytokines. Further studies are needed to evaluate the role of SOCS1 and SOCS3 in cytokine regulation in CRS and to investigate their potential therapeutic implications.

Clinical implications: Current data support the idea that cytokine activity deregulated by SOCS is involved in the pathogenesis of inflammatory diseases.

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