

Expression of SOCS1 and SOCS3 Is Altered in the Nasal Mucosa of Patients with Mild and Moderate/Severe Persistent Allergic Rhinitis

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Key Words

Allergic nasal mucosa · Healthy nasal mucosa · Persistent allergy · Rhinitis · SOCS1 · SOCS3

Abstract

Background: The aim of this study was to evaluate the role of suppressor of cytokine signaling (SOCS) molecules, SOCS1 and SOCS3, which act as negative regulators of cytokine signaling in various allergic diseases, in patients with mild and moderate/severe persistent allergic rhinitis. **Methods:** The expression and distribution pattern of SOCS1 and SOCS3 were analyzed in nasal mucosa and peripheral blood mononuclear cells (PBMC) of healthy controls, and patients with mild and moderate/severe persistent allergic rhinitis using RT-PCR, immunohistochemistry and Western blotting. IL-4, IL-13, IL-15 and IFN- γ expression was also analyzed in the nasal mucosa of each individual using RT-PCR and Western blotting. **Results:** SOCS1 and SOCS3 mRNA and protein expression was significantly increased in the nasal mucosa and PBMC of patients with mild and moderate/severe persistent allergic rhinitis compared with healthy controls. In healthy and allergic nasal mucosa, they were commonly localized to the epithelium, submucosal glands and endothelium, showing stronger staining intensity in mild and moderate/severe persistent allergic nasal mucosa than in healthy nasal mu-

cosa. Tissue levels of IL-4 and IL-13 were increased in moderate/severe persistent allergic nasal mucosa whereas IL-15 and IFN- γ were decreased in moderate/severe persistent allergic nasal mucosa. **Conclusions:** Upregulation of SOCS1 and SOCS3 in mild and moderate/severe persistent allergic rhinitis suggests that SOCS proteins may be important regulators in the pathogenesis of allergic rhinitis and play a role as molecular determinants of allergic rhinitis persistence.

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Introduction

Allergic rhinitis, which is characterized by chronic inflammation, eosinophilic infiltration, nasal hyperresponsiveness to non-specific stimuli, hyperplasia of goblet cells and subepithelial fibrosis, is an important, clinically prevalent disease worldwide [1]. Clinically, allergic rhinitis is characterized by bothersome symptoms, including rhinorrhea, nasal obstruction, sneezing and nasal itching. Currently, allergic rhinitis is more commonly classified as intermittent or persistent allergic rhinitis based on the frequency and duration of symptoms irrespective of the nature of allergen exposure [2]. Despite an increasing knowledge of its pathogenesis, the mechanisms underlying the induction and persistence of aller-

gic rhinitis are largely unknown. According to some reports, the symptoms of allergic rhinitis may result from the allergen-induced release of a variety of proinflammatory mediators, including cytokines from various inflammatory cells [3, 4]. After nasal exposure to an allergen, infiltration of inflammatory cells, e.g. activated eosinophils and Th2 cells, into the nasal mucosa is induced by cytokines and other mediators [5]. Cytokines, e.g. IL-4, IL-5 and IL-13, are produced mainly in Th2 cells, playing a pivotal role in the pathophysiology of allergic inflammation [5]. Thus, cytokines are thought to play an important pathogenic role in allergic rhinitis.

Cytokines regulate many physiologic responses and homeostasis, thus influencing survival, proliferation, differentiation and functional activity of the cells of the immune system, as well as the cells of most other organ systems. It is now clear that sustained and/or excessive cytokine activation can lead to local and/or systemic disease. Thus, under physiologic conditions, as well as in pathologic states, cytokine functions are strictly controlled to prevent their overreaction. Accordingly, several mechanisms have been reported to modulate cytokine signaling to prevent the overreaction of inflammatory mediators [6, 7]. Specifically, recent studies have indicated that suppressor of cytokine signaling (SOCS), a family of molecules that act as negative regulators for cytokine signaling, modulate cytokine signaling [7]. The SOCS protein family consists of 8 members (cytokine-induced SH-2 containing protein and SOCS1–SOCS7) [7]. Particularly SOCS1 and SOCS3 control the magnitude and duration of JAK/STAT signaling through several mechanisms in response to a wide range of cytokines [7]. Recently, SOCS proteins have been implicated in the inflammatory response of various organs, including the lung and skin [8, 9]. Human studies have suggested that increased SOCS1 expression is linked to asthma susceptibility via a reduction in the IFN- γ -mediated Th1 response [8]. SOCS1 and SOCS3 overexpression has been shown in peripheral blood mononuclear cells (PBMC) from patients with rheumatoid arthritis [10]. SOCS3 is preferentially expressed in Th2 cells, and SOCS3 levels are increased in patients suffering from asthma and atopic dermatitis [8, 9]. Thus, considering the fact that SOCS is a pivotal regulator for cytokine signaling, an alterations in SOCS1 and SOCS3 expression may play an important role in the pathogenesis of allergic rhinitis. Although all of these properties are relevant to the pathogenesis of allergic rhinitis, the precise involvement of SOCS proteins in patients with allergic rhinitis has not been sufficiently evaluated. To elucidate the role of SOCS proteins in patients

with allergic rhinitis, the level of expression and the distribution pattern of SOCS1 and SOCS3 in allergic rhinitis have to be defined.

The present study was performed to determine the expression level and distribution pattern of SOCS1 and SOCS3 in healthy nasal, and mild and moderate/severe persistent allergic nasal mucosa to evaluate the possible effects of SOCS1 and SOCS3 on the pathogenesis of allergic rhinitis. In addition, SOCS1 and SOCS3 expression was evaluated in PBMC obtained from the patients. We also evaluated the role of IL-4, IL-13, IL-15 and IFN- γ in the pathogenesis of allergic rhinitis, which may be direct or indirect targets of SOCS proteins.

Subjects and Methods

Thirty patients with mild persistent allergic rhinitis, 29 patients with moderate/severe persistent allergic rhinitis, and 30 healthy control subjects were enrolled in the current study. Patients undergoing augmentation rhinoplasty were considered healthy controls and were excluded if they had a history of nasal infection or allergy, smoking or ongoing drug treatment. All patients with allergic rhinitis had typical symptoms of perennial nasal allergy, nasal eosinophilia, and a positive skin test or positive RAST to house dust mites, as well as positive reactions on provocation tests. In addition, serum total IgE was determined by enzyme-linked immunosorbent assay (CAP system). The detectable range of the assay for total IgE was 2–2,000 IU/ml. Allergic patients with exacerbation of upper respiratory tract infection during the 6 weeks preceding the study were not included in the study. Other exclusion criteria were current smoking, a medical history of asthma, recurrent infections of the respiratory tract, and use of topical or oral corticosteroids, leukotriene modifiers and antihistamines during the 8 weeks that preceded the study.

Thirty samples of healthy nasal mucosa were obtained from the inferior turbinates of 30 healthy control subjects (22 men and 8 women; age range, 20–44 years) who had normal nasal mucosa and were admitted for augmentation rhinoplasty. A mucosal biopsy sample was obtained about 2 cm posterior to the anterior edge of the inferior turbinate using Blakesley forceps with a cup diameter of 4 $\times$ 10 mm.

Fifty-nine patients with persistent allergic rhinitis were classified according to disease severity, and severity of rhinitis was classified according to allergic rhinitis and its impact on asthma reported as mild or moderate/severe [2]. Patients with moderate/severe rhinitis had one or more of the following symptoms: sleep disturbance, impairment in daily activities, leisure and/or sports and school or work, or troublesome symptoms. Patients with mild rhinitis had none of these symptoms.

Nasal biopsy specimens were also obtained from the inferior turbinate of all patients with mild (22 men and 8 women; age range, 24–47 years) and moderate/severe persistent allergic rhinitis (20 men and 9 women; age range, 22–45 years) using Blakesley forceps with a cup diameter of 4 $\times$ 10 mm under local anesthesia. A mucosal biopsy sample was also taken about 2 cm posterior to the anterior edge of the inferior turbinate. Prior to obtaining the

tissue specimens, the protocol and informed consent form were approved by the Institutional Review Board for Human Studies our Institution, and all participants gave written informed consent.

All tissue samples were divided into three portions. The first and second portions were frozen in liquid nitrogen and stored at -70°C for RNA and protein isolation. For immunohistochemistry, the third sample was immersed overnight in a freshly prepared fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4). All of the samples were then dehydrated in a graded series of ethanol to xylene and embedded in paraffin wax.

Whole blood samples were collected from 30 patients with mild persistent allergic rhinitis, 29 patients with moderate/severe persistent allergic rhinitis, and 30 healthy control subjects, and then Ficoll-Hypaque density centrifugation was immediately performed to isolate PBMCs. Total RNA and protein were extracted from PBMC for RT-PCR and Western blot analysis.

RT-PCR and Real-Time RT-PCR Analysis

Total RNA (1 μg) from each sample was reverse transcribed in 20 μl of a reaction mixture containing 2.5 U of Moloney murine leukemia virus reverse transcriptase (RT; GIBCO BRL, Grand Island, N.Y., USA) and 50 pmol of random hexanucleotides at 42°C for 60 min. RNA integrity and the success of the reverse transcription reaction were monitored by PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. The primer sequences of each gene used in the study are listed in table 1. PCR products were analyzed by electrophoresis in 1.5% agarose gel stained with ethidium bromide.

Real-time PCR was carried out using an iCycler (BioRad Laboratories, Hercules, Calif., USA). Each PCR included 50 ng cDNA, 12.5 μl iTAQ SYBR supermix and 200 nM of primers. PCR conditions for 40 cycles were 95°C denaturation for 30 s, 59°C annealing for 30 s and 72°C extension for 30 s. GAPDH was used as a control housekeeping gene to normalize for transcription and amplification variations among samples. All PCR were performed in duplicate. Melting curve analysis was used to control for amplification specificity. Target gene expression is expressed as the fold increase and decrease relative to the expression of GAPDH. The mean value of the replicates for each sample was calculated and expressed as the cycle threshold (C_t). The amount of gene expression was then calculated as the difference (ΔC_t) between the C_t value obtained for the target gene and the C_t value obtained for GAPDH. Fold change in the target gene mRNA expression was determined as $2^{-\Delta\Delta C_t}$. The Kruskal-Wallis test was conducted to determine if the observed difference in the mean fold change among healthy, and mild and moderate/severe persistent allergic nasal mucosa was statistically significant, with $p < 0.05$ as threshold.

Immunohistochemical and Western Blot Analysis

Immunohistochemical staining was performed 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, Inc., Santa Cruz, Calif.,

Table 1. Sequences of PCR primers

Primer	Size bp	Sequence
SOCS1	109	S: 5'-CGCCCTTAGCGTGAAGAT-3' AS: 5'-CTCGAAGAGGCAGTCGAAG-3'
SOCS3	83	S: 5'-TTCAGTCCAAGAGCCGAGTA-3' AS: 5'-GTCACTGCGCTCCAGTAGAA-3'
IL-4	169	S: 5'-TTGCTGCCTCCAAGAACACAACCTG-3' AS: 5'-TTCCTGTCGAGCCGTTTCAGGAAT-3'
IL-13	120	S: 5'-TGGTCAACATCACCCAGAACCAGA-3' AS: 5'-AGCCTGACACGTTGATCAGGGATT-3'
IL-15	183	S: 5'-GTCTAGAGCCAACCTGGGTGAATGT-3' AS: 5'-CTTGCATCTCCGGACTCAAGTGAA-3'
IFN- γ	182	S: 5'-TGCAGGTCAATTCAGATGTAGCGGA-3' AS: 5'-TGTCTTCTTTCGATGGTCTCCACACTC-3'
GAPDH	122	S: 5'-ATCATCCCTGCCTCTACTGG-3' AS: 5'-GTCAGGTCCACCACTGACAC-3'

USA). The color was developed using 3,3'-diaminobenzidine. The negative immunohistochemical control procedure included omission of the primary antibodies and their replacement with normal rabbit IgG in appropriate concentrations. For peptide blocking studies, a series of 2 sections of all samples were incubated with each antibody, which has been preincubated for 1 h with peptides 119–211 mapping within an internal region of SOCS1 and peptides 87–189 of SOCS3 of human origin. Five sections and four random fields per section were analyzed for each subject. In addition, to assess the staining intensity of SOCS1 and SOCS3, all tissue sections were independently examined by two different investigators. The intensity of SOCS1 and SOCS3 expression was classified into four degrees as follows: –, negative; +, weakly positive; ++, moderately positive, and +++, strongly positive. Quantitative analysis of the intensities for each patient was calculated as the mean of the total score in the tissue specimen.

For Western blot analysis, frozen tissue sections or PBMC were crushed and vigorously vortexed in a buffer solution containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl and a proteinase inhibitor cocktail. The extracted protein (50 μg) was resuspended in sodium dodecyl sulfate sample buffer and boiled for 5 min. Equal amounts of total protein were separated on 12% sodium dodecyl sulfate polyacrylamide gels and transferred to Immobilon (Millipore, Bedford, Mass., USA). The blots were blocked for 1 h at room temperature with PBS-T containing 1% skim milk, then incubated with a 1:250 dilution of anti-SOCS1 or a 1:250 dilution of anti-SOCS3 antibody or a 1:500 dilution of anti-IFN- γ , anti-IL-15, anti-IL-13 or anti-IL-4 (all 1:500 dilutions of antibodies from Santa Cruz Biotechnology) in PBS-T overnight at room temperature. Antibody reactions were detected using the ECL detection kit [GE Healthcare (Amersham Biosciences)], followed by detection of chemiluminescence on X-ray film. As an internal control, β -actin expression was analyzed in parallel blots using the β -actin antibody (Sigma, St. Louis, Mo., USA). The intensity of detected bands was quantified using Scion Image Beta 4.0.2

Table 2. Clinical characteristics of the study cohort

Characteristics	Healthy nasal mucosa (n = 30)	Allergic rhinitis	
		mild (n = 30)	moderate/severe (n = 29)
Females/males	8/22	8/22	9/20
Age, years	33.7 ± 6.2	35.4 ± 7.9	33.9 ± 6.8
Rhinitis duration, years	–	11.78 ± 1.3	12.42 ± 1.73
Serum total IgE, IU/ml	35.34 ± 15.20	505.23 ± 202.85	527.04 ± 250.76

(Scion Corporation, Frederick, Md., USA). The relative intensities of each protein signal were obtained by dividing the intensities of each protein signal by the β -actin signal.

Statistical Analysis

Statistical analyses were carried out using SPSS for Windows (version 16.0.0; SPSS, Chicago, Ill., USA). Age differences in the three groups were calculated by one-way ANOVA, and Bonferroni post hoc correction was applied. The duration of rhinitis between the mild and moderate/severe persistent rhinitis groups was statistically evaluated by the Mann-Whitney U test. The Kruskal-Wallis test was performed to establish statistically significant differences in values obtained from the nasal mucosa of healthy controls and the patients with mild or moderate/severe persistent allergic rhinitis, including the data evaluated by real-time PCR and Western blot analysis. The level of significance was set at $p < 0.05$. Mann-Whitney's post hoc tests were done to determine intergroup differences.

Results

The characteristics of the patients with mild and moderate/severe persistent allergic rhinitis and healthy controls are shown in table 2. Age was similarly distributed among the healthy controls and the patients with mild and moderate/severe persistent allergic rhinitis. The duration of disease was not significantly different between patients with mild and moderate/severe persistent allergic rhinitis (table 2). However, serum total IgE levels were increased in mild and moderate/severe persistent allergic rhinitis compared with healthy nasal mucosa (table 2).

SOCS1, SOCS3 and GAPDH mRNA expression was determined by RT-PCR and real-time PCR in total RNA preparations isolated from healthy, and mild and moderate/severe persistent allergic nasal mucosa, and PBMC of healthy controls and patients. As shown in figure 1a and 2a, the PCR products of the expected size corresponding to 109 bp for SOCS1, 83 bp for SOCS3 and 122 bp for GAPDH were detected in healthy, and mild and moderate/severe persistent allergic nasal mucosa, and PBMCs of each individual, and had 100% sequence homology with the published sequence. Negative control reactions with-

out RT in the cDNA synthesis process showed no bands, indicating that there was no contamination by genomic DNA in the RNA samples (data not shown). Real-time RT-PCR showed that the level of expression of SOCS1 and SOCS3 mRNA was significantly increased in nasal mucosa and PBMC of patients with mild and moderate/severe persistent allergic nasal mucosa compared with healthy nasal mucosa (fig. 1b; $p < 0.05$; fig. 2a, c). However, SOCS1 and SOCS3 mRNA expression was not significantly different between mild and moderate/severe persistent allergic nasal mucosa (fig. 1b) and between PBMC of patients with mild and moderate/severe persistent allergic rhinitis (fig. 2a, c).

IL-4, IL-13, IL-15 and IFN- γ mRNA expression was also determined in nasal mucosa of healthy controls and patients with mild and moderate/severe persistent allergic rhinitis using RT-PCR and real-time RT-PCR. The results showed that IL-4, IL-13, IL-15 and IFN- γ mRNA could be detected in nasal mucosa from healthy controls and patients with mild and moderate/severe persistent allergic rhinitis (fig. 1a). IL-4 and IL-13 mRNA expression was increased in moderate/severe persistent allergic nasal mucosa compared with healthy controls and mild persistent allergic nasal mucosa (fig. 1b). However, IL-15 and IFN- γ mRNA expression was decreased in moderate/severe persistent allergic nasal mucosa compared with healthy controls and mild persistent allergic nasal mucosa (fig. 1b).

To determine the expression and distribution pattern of SOCS1 and SOCS3 in healthy and allergic nasal mucosa, tissue sections were analyzed by immunohistochemistry. Immunohistochemical staining showed a homologous pattern in all samples of nasal mucosa: healthy nasal mucosa, and mild and moderate/severe persistent allergic nasal mucosa (fig. 3). As summarized in table 3, in healthy, and mild and moderate/severe persistent allergic nasal mucosa, SOCS1 and SOCS3 were exclusively localized to the superficial epithelium, submucosal glands and vascular endothelium. Their staining intensity was stronger in mild and moderate/severe persistent

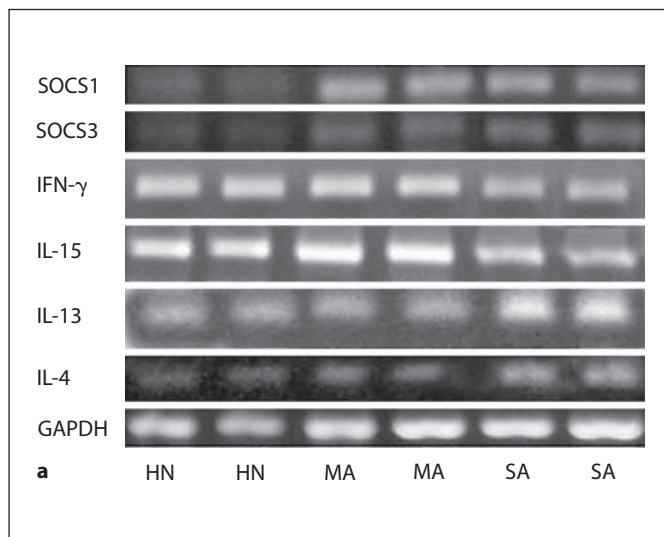
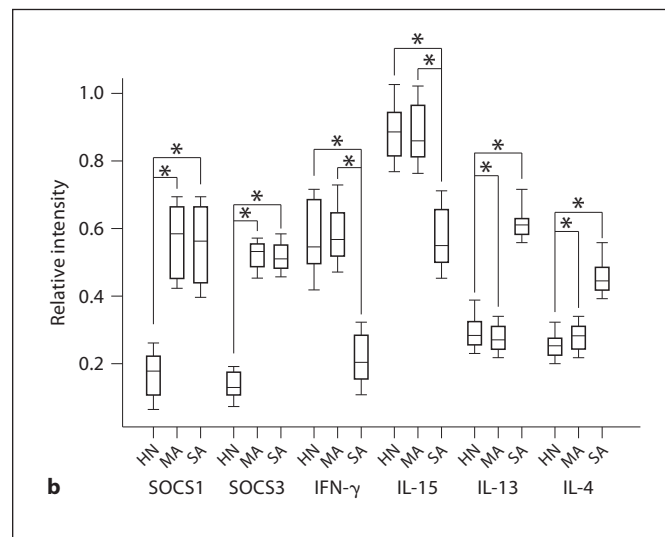


Fig. 1. a Analysis of SOCS1, SOCS3, IFN- γ , IL-15, IL-13 and IL-4 mRNA levels in healthy (HN), and mild (MA) and moderate/severe persistent allergic nasal mucosa (SA). **b** In real-time RT-PCR, SOCS1 and SOCS3 expression was increased in mild (MA) and moderate/severe persistent allergic nasal mucosa (SA) compared with healthy nasal mucosa (HN). IFN- γ and IL-15 mRNA expression was decreased in moderate/severe persistent allergic nasal mucosa in comparison to healthy and mild persistent allergic nasal mucosa. IL-13 and IL-4 mRNA levels were increased in mod-



erate/severe persistent allergic nasal mucosa. The Kruskal-Wallis test was used to determine significant differences (* $p < 0.05$) in SOCS1, SOCS3, IFN- γ , IL-15, IL-13 and IL-4 mRNA expression among healthy nasal mucosa ($n = 30$), and mild ($n = 30$) and moderate/severe persistent allergic nasal mucosa ($n = 29$). The data shown are the relative ratios measured by real-time PCR. Data are medians, and 25th and 75th percentiles. The bottom and top boxes are the lower and upper quartiles, respectively, and the horizontal lines between the top and bottom boxes are medians.

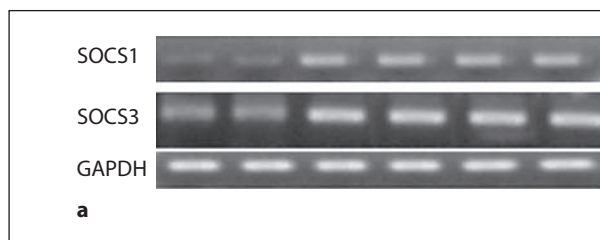
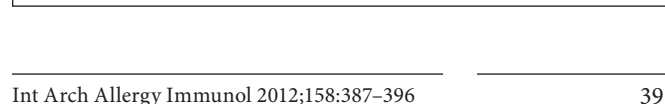
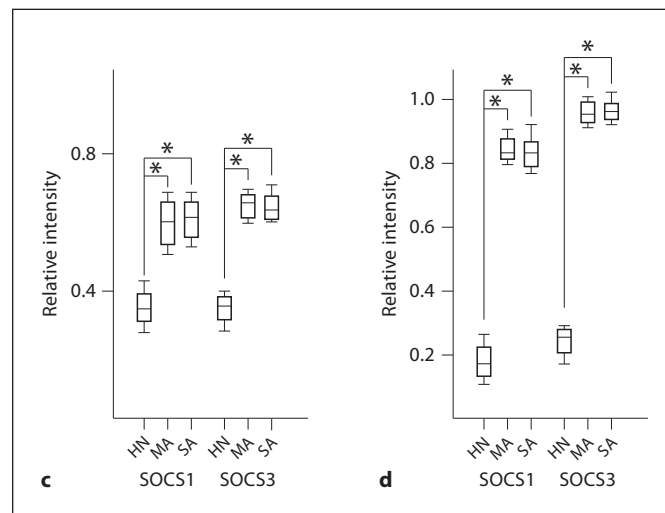
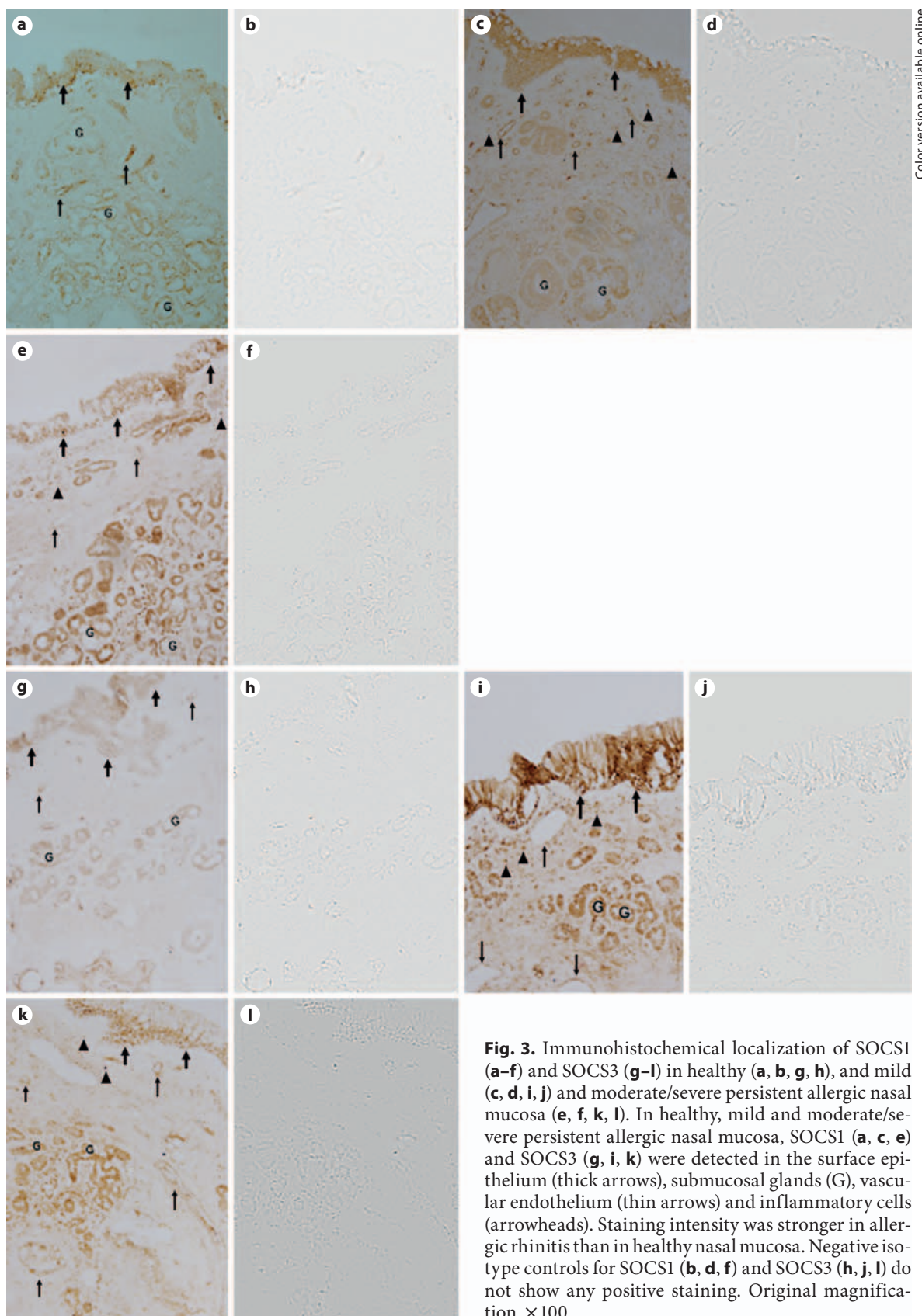


Fig. 2. a RT-PCR, real-time PCR (**c**), and Western blot analysis (**b**, **d**) of SOCS1 and SOCS3 in PBMCs of healthy controls and patients with mild and moderate/severe persistent allergic rhinitis. Real-time RT-PCR results demonstrated increased expression levels of SOCS1 and SOCS3 in PBMCs of patients with mild and moderate/severe persistent allergic rhinitis compared with healthy nasal mucosa (**c**). Western blot also showed the increased expression level of SOCS1 and SOCS3 proteins in PBMCs of patients with mild and moderate/severe persistent allergic rhinitis (**b**, **d**). The bottom and top of box are the lower and upper quartiles, respectively, and the band near the middle of the box is the median. HN = Healthy controls; MA = mild persistent allergic rhinitis; SA = moderate/severe persistent allergic rhinitis. * $p < 0.05$.





Color version available online

Fig. 3. Immunohistochemical localization of SOCS1 (**a–f**) and SOCS3 (**g–l**) in healthy (**a, b, g, h**), and mild (**c, d, i, j**) and moderate/severe persistent allergic nasal mucosa (**e, f, k, l**). In healthy, mild and moderate/severe persistent allergic nasal mucosa, SOCS1 (**a, c, e**) and SOCS3 (**g, i, k**) were detected in the surface epithelium (thick arrows), submucosal glands (G), vascular endothelium (thin arrows) and inflammatory cells (arrowheads). Staining intensity was stronger in allergic rhinitis than in healthy nasal mucosa. Negative isotype controls for SOCS1 (**b, d, f**) and SOCS3 (**h, j, l**) do not show any positive staining. Original magnification $\times 100$.

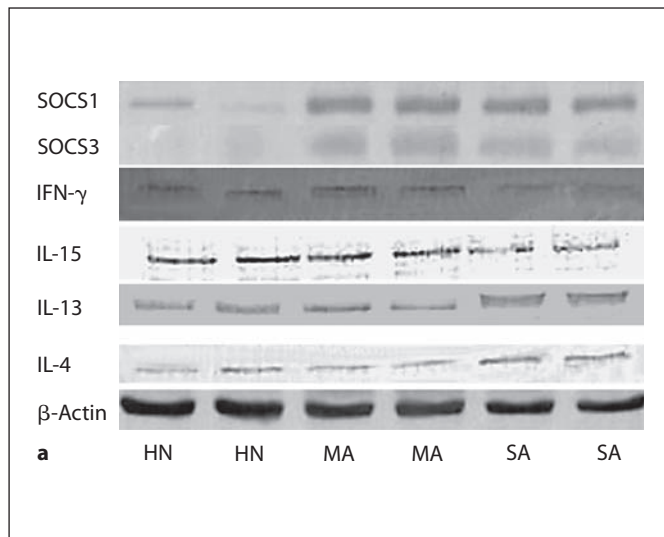
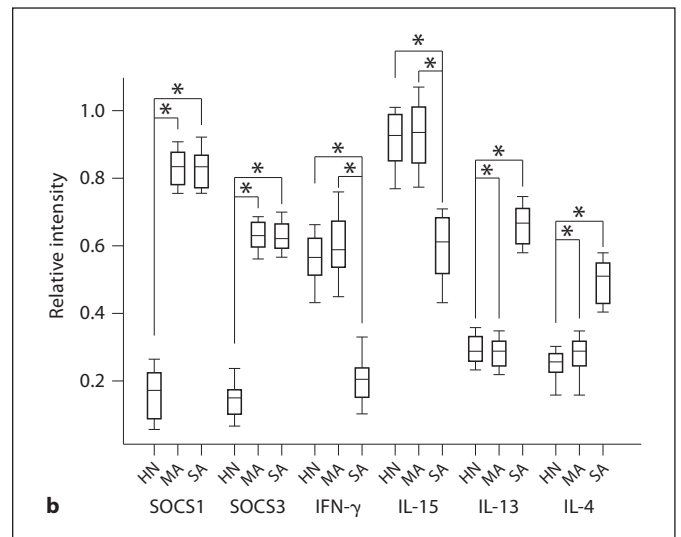


Fig. 4. a Western blot analysis of SOCS1, SOCS3, IFN-γ, IL-15, IL-13 and IL-4 in healthy (HN), and mild (MA) and moderate/severe persistent allergic nasal mucosa (SA). **b** Bands were quantified by densitometric scanning, and the relative amount of each



protein was calculated with respect to the internal control (β-actin). The bottom and top boxes are the lower and upper quartiles, respectively, and the horizontal lines between the bottom and top boxes are medians. * p < 0.05.

allergic nasal mucosa compared with healthy nasal mucosa (fig. 3). In addition, SOCS1- and SOCS3-positive inflammatory cells were commonly increased in both types of allergic nasal mucosa in comparison with healthy nasal mucosa. These increases were completely inhibited when the antibodies were preincubated in the presence of synthetic peptides or in the negative immunohistochemical control procedure including omission of the primary antibodies and their replacement by normal rabbit IgG in appropriate concentrations (fig. 3).

Western blot analysis with SOCS1 and SOCS3 antisera detected proteins in healthy, and mild and moderate/severe persistent allergic nasal mucosa and PBMC (fig. 2a, 4a). The relative abundance of SOCS1 and SOCS3 with respect to β-actin was significantly increased in nasal mucosa and PBMC of patients with mild and moderate/severe persistent allergic rhinitis compared with healthy controls, demonstrating that SOCS1 and SOCS3 expression was increased in mild and moderate/severe persistent allergic rhinitis compared with healthy controls (fig. 2d, 4b). There were no differences in the expression of SOCS1 and SOCS3 protein between nasal mucosa of patients with mild and moderate/severe persistent allergic rhinitis and between PBMC of both patient groups (fig. 2d, 4b). IFN-γ and IL-15 expression was decreased in nasal mucosa of patients with moderate/severe persistent allergic rhinitis compared with healthy controls and mild

Table 3. Distribution pattern of SOCS1 and SOCS3 in healthy and mild and moderate/severe persistent allergic nasal mucosa

	Healthy nasal mucosa		Persistent allergic nasal mucosa			
	SOCS1	SOCS3	mild SOCS1	mild SOCS3	moderate/severe SOCS1	moderate/severe SOCS3
Epithelium	+	+	+++	+++	+++	+++
Submucosal gland	+	+	+++	+++	+++	+++
Endothelium	+	+	+++	+++	+++	+++
Inflammatory cells	+	+	+++	+++	+++	+++

persistent allergic patients, whereas IL-13 and IL-4 expression was decreased in moderate/severe persistent allergic nasal mucosa in comparison to healthy controls and mild persistent allergic nasal mucosa (fig. 4b).

Discussion

In an effort to identify cytokines that may be important in allergic rhinitis, the majority of studies have focused on investigating cytokine levels in nasal fluid and mucosa in patients with allergic rhinitis [11, 12]. SOCS proteins are at the crossroads of multiple immunological

and inflammatory pathways and play an important role in maintaining organ homeostasis by preventing harmful cytokine responses in various organs [13]. Thus, we considered it necessary to perform a detailed analysis of SOCS expression in healthy controls and patients with allergic rhinitis to characterize factors that may alter cellular responsiveness to cytokines in allergic rhinitis. Our study has shown that the expression of SOCS1 and SOCS3 is upregulated in mild and moderate/severe persistent allergic nasal mucosa compared with healthy nasal mucosa at mRNA and protein levels. Immunohistochemistry showed that SOCS1 and SOCS3 are commonly localized to the superficial epithelium, submucosal glands and vascular endothelium in healthy controls, and patients with mild and moderate/severe persistent allergic nasal mucosa. However, the staining intensities were also increased in mild and moderate/severe persistent allergic nasal mucosa compared with healthy nasal mucosa. In addition, many inflammatory cells immunopositive for SOCS1 and SOCS3 were found in both types of allergic nasal mucosa. Taken together, the present results indicate that SOCS1 and SOCS3 are constitutively expressed in healthy human nasal mucosa and upregulated in nasal mucosa of patients with mild and moderate/severe persistent allergic nasal mucosa, suggesting that SOCS1 and SOCS3 may play an important role in the pathophysiology of allergic rhinitis. This is the first study comparing the level of expression and pattern of distribution of SOCS1 and SOCS3 in mild and moderate/severe persistent allergic rhinitis with healthy nasal mucosa, and our results may be useful in the development of specific target treatment strategies for the management of these patients.

Evidence is emerging in favor of the involvement of SOCS1 and SOCS3 in human autoimmune and other allergic diseases. For example, the levels of SOCS1 and SOCS3 are significantly increased in PBMC from patients with rheumatoid arthritis compared with healthy volunteers [10]. SOCS3 is increased in the intestinal mucosa derived from patients with Crohn's disease and ulcerative colitis [14, 15]. SOCS3 is highly expressed at the disease site of allergic conjunctivitis [16]. Further, SOCS1 and SOCS3 expression is also elevated in the skin of patients with allergic contact dermatitis [17]. Consistent with these results, the present study showed that SOCS1 and SOCS3 mRNA and proteins are increased in nasal mucosa and PBMC of patients with mild and moderate/severe persistent allergic rhinitis compared with healthy nasal mucosa, suggesting that SOCS1 and SOCS3 may play an important role in controlling allergic rhinitis.

However, the mechanism that mediates the upregulation of SOCS1 and SOCS3 in allergic rhinitis remains unknown. One possible mechanism is that the elevated levels of Th2 cytokines in individuals with allergic rhinitis 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 [7]. Indeed, allergic rhinitis is related to an enhancement in Th2 lymphocyte responses, with increased levels of Th2-type cytokines, e.g. IL-4, IL-5 and IL-13, in nasal mucosa [4]. The results of the present study also demonstrated that IL-4 and IL-13 expression was increased in moderate/severe persistent allergic rhinitis.

Studies investigating cytokine expression showed that increased levels of SOCS1 and SOCS3 could suppress cytokine signaling and potentially prevent the deleterious actions of proinflammatory cytokines, such as IL-6, IL-12, IL-15, GM-CSF and IFN- γ [7]. Further, high levels of these SOCS proteins could inhibit the beneficial effects of anti-inflammatory cytokines, participating in the negative feedback regulation of the JAK/STAT signal transduction pathway induced by cytokine signaling [7]. These data appear to be contradictory to the results of the current study, which found a positive association between SOCS1 or SOCS3 and allergic status. Our results can be interpreted by the following data. The enhanced expression of SOCS1 and SOCS3 may result in inhibition of IFN- γ signaling, skewing towards Th2 polarization and exacerbating allergic rhinitis. Indeed, it has been clearly shown that Th1 downregulation by SOCS1 and SOCS3 may not only result in an accelerated Th2 response but also in Th2-related diseases like allergy [17]. SOCS1 is an essential physiologic negative regulator of IFN- γ signaling that is crucial for the induction of appropriate immune responses. SOCS3 also represses IFN- γ signaling but less than SOCS1 [17]. Allergic rhinitis is thought to arise from an imbalance in Th1-Th2 immune regulation, and IFN- γ -producing Th1 cells have been suggested to protect against allergic responses by attenuating the activity of Th2 effector cells [18]. Therefore, in association with SOCS1 and SOCS3 upregulation, a reduced number of IFN- γ -producing Th1 cells may show a strong correlation to the development of allergic sensitization, implicating SOCS1 and SOCS3 as candidate proteins involved in allergic rhinitis. This suggestion is supported by evidence demonstrating that IFN- γ mRNA was expressed at significantly higher levels in control than in sensitized allergic rats [19]. Indeed, IFN- γ levels were not elevated

in allergic rhinitis patients [20]. The present results also showed that the expression levels of IL-15 and IFN- γ are decreased in the mucosa of patients with moderate/severe persistent allergic rhinitis compared with healthy nasal mucosa.

The present data show that SOCS1 and SOCS3 expression is significantly higher in the epithelium, submucosal gland and vascular endothelium, and that inflammatory cells infiltrated the allergic nasal mucosa, suggesting that these target cells in human nasal mucosa are the source of SOCS1 and SOCS3 protein. A number of studies have revealed that various cytokines are also produced in nasal mucosa cells of patients with allergic rhinitis [21, 22]. Considering these results, one particularly compelling, but underappreciated, aspect of the involvement of SOCS in allergic rhinitis is the bidirectional interplay between SOCS and cytokines in these cells composing the nasal mucosa. The modified responses of these cells may promote the inflammatory process which is responsible for the pathogenesis of allergic rhinitis. However, little is known regarding the role of SOCS1 and SOCS3 proteins in allergic rhinitis. Daegelmann et al. [23] found an association between increased SOCS1 expression, allergic sensitization and Th2 polarization. Therefore, enhanced SOCS1 expression may result in inhibition of IFN- γ signaling, skewing towards Th2 polarization and exacerbation of allergic rhinitis. Conversely, the elevated levels of Th2 cytokines found in patients with allergic rhinitis may result in cytokine-induced elevation in SOCS1 as part of the normal transcriptional response. On the other hand, the enhanced expression of SOCS3 may also promote allergic responses, because a recent analysis by Seki et al. [24] indicated that transgenic SOCS3 expression in T cells inhibits Th1 development and promotes Th2 development. Indeed, they also described that increased SOCS3 expression in T cells is correlated with the severity of human allergic diseases such as asthma and atopic dermatitis [24]. Additional studies using antisense oligonucleotides or specific transcriptional inhibitors are required to determine the role of these proteins in the modulation of host response associated with allergic rhinitis.

It is likely that nasal inflammation persists longer in patients with persistent allergic rhinitis than in those with intermittent allergic rhinitis, and should be more severe in patients with moderate/severe than mild symptoms. Actually, several studies have found a correlation between symptom severity and several mediators [25, 26]. Unfortunately, the current study did not find differences in the expression level of SOCS1 and SOCS3 between

mild and moderate/severe persistent allergic rhinitis patients. It is possible that symptom frequency and duration, rather than symptom severity, may alter SOCS1 and SOCS3 expression in patients with allergic rhinitis. This suggestion is supported by a study showing that moderate/severe persistent allergic rhinitis is characterized by a significantly greater eosinophilic and predominantly Th2 cell-mediated nasal inflammatory profile compared to intermittent allergic rhinitis [27]. However, our study did not evaluate patients with intermittent allergic rhinitis, and future studies investigating SOCS1 and SOCS3 expression in these patients may be promising.

In conclusion, we have shown increased levels of expression and distinctive distribution patterns for SOCS1 and SOCS3 in mild and moderate/severe persistent allergic nasal mucosa compared with healthy nasal mucosa. These findings provide the basis for further studies on the role of SOCS protein activation in patients with allergic rhinitis by providing a baseline of SOCS1 and SOCS3 protein distribution in healthy, and mild and moderate/severe persistent allergic nasal mucosa.

Acknowledgments

This work was supported by a grant-in-aid for scientific research from the Communication Disorders Center, Korea University, Korea, and the Basic Science Research Program of the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010-0004094). This research was also supported by a grant from Korea University.

Disclosure Statement

There are no conflicts of interest.

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