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ORIGINAL ARTICLE

Interleukin-19 Downregulates Interleukin-4-Induced Eotaxin Production in Human Nasal Fibroblasts

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ABSTRACT

Background: Interleukin-19 (IL-19), a member of the IL-10 family, is characterized as the cytokine suppressing the release and function of several proinflammatory cytokines. For regulation of local reaction in allergic rhinitis (AR), IL-19 might play an especially important role.

Methods: We examined effects of IL-19 on IL-4-induced eotaxin production by human nasal fibroblasts. Early receptor-mediated events (expression of the suppressors of cytokine signaling (SOCS) and phosphorylation of signal transducer and activator of transcription 6 [STAT6]) by IL-19 was examined. Knockdown methods by RNAi were administered to investigate the involvement of those signal transductions.

Results: Pretreatment with IL-19 downregulates IL-4-induced eotaxin production, but not interferon-γ (IFN-γ)-induced RANTES. Pretreatment with IL-19 suppressed the IL-4-induced STAT6 phosphorylation. The IL-19 induced SOCS-1, but not SOCS-3 or SOCS-5. The SOCS-1 knockdown by RNAi diminished pretreatment with IL-19-induced down-regulation of eotaxin production.

Conclusions: These results suggest that IL-19 down-regulates IL-4-induced eotaxin production via SOCS-1 in human nasal fibroblasts. In non-hematopoietic cells in AR, IL-19 might be an immunosuppressive factor.

KEY WORDS

eotaxin, human nasal fibroblast, Interluekin-19, SOCS-1, STAT6

INTRODUCTION

As an important immunoregulatory cytokine, IL-10 is known to have multiple biologic effects on different cell types. The IL-10 family includes IL-19, IL-20, IL-22, IL-24, and IL-26.[1-3] In fact, IL-19 is detected by human monocytes, B cells, and T cells.[3] Inflammatory stimulation such as that by lipopolysaccharide (LPS) or GM-CSF treatment induces IL-19 mRNA by monocytes. Pre-priming monocytes with IL-4 enhances the induction of IL-19 by LPS-treatment, although pre-priming with IFN-γ apparently prevents LPS-induced IL-19 expression.[4] Furthermore, in whole peripheral blood mononuclear cells cultured with Con-A, IL-19 up-regulated IL-4 and down-regulated IFN-γ dose-dependently.[4] The serum levels of IL-19 in children with atopic asthma were twice those of healthy children.[5] The *in vitro* treatment of IL-19 induced IL-4, IL-5, IL-10, and IL-13 production by activated T cells.[5] Long-term exposure of naïve T cells to IL-19 down-regulated the differentiation to IFN-γ-producing T cells, but up-regulated differentiation to IL-4 and IL-13 producing T cells.[6] These results suggest that IL-19 positively contributes to Th2 response.

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Reportedly, nasal fibroblasts are not passive players in the immune system.[7] Fibroblasts, long considered mainly as constituting a physical barrier, have recently been reported as important modulators of local inflammation because of their capacity to release various pro-inflammatory mediators including eotaxin, RANTES, thymus, and activation-regulated chemokine (TARC), GM-CSF, and IL-8.[8-10] Eotaxin and RANTES are implicated in the recruitment and enhanced survival of eosinophils[11,12]; TARC causes selective migration of Th2 cells.[10] We showed previously that pro-inflammatory cytokines and IFN-γ induced production of RANTES and that IL-4 induced eotaxin by nasal fibroblasts.[13]

Recently it was reported that vascular smooth muscle cells[14] and airway epithelial cells[15] express IL-19, although IL-19 expression had been ascribed to be restricted to immune cells. Nevertheless, the influence of IL-19 on the structural cells (ex fibroblasts, epithelial cells) remains unclear.

In this study, we investigated the effect of IL-19 on chemokine production by human nasal fibroblasts. Results show that IL-19 inhibited IL-4-induced eotaxin production by fibroblasts, which was unexpected because IL-19 has been shown to be an inducer of Th2 cytokine (Th2 immunity).[3-6] Actually, the Th2/Th1 shift induced by IL-19 might depend on the cell type. Fulfillment of IL-19 function requires the induction of suppressors of cytokine signaling-1 (SOCS-1) in fibroblasts.

METHODS

REAGENTS

Recombinant human IL-4, IL-19, and IFN-γ were obtained from PeproTech EC Ltd. (London, UK). The eotaxin and RANTES kits were purchased from Biosource International Inc. (Camarillo, CA, USA).

HUMAN NASAL MUCOSA-DERIVED FIBROBLAST CELL CULTURE AND STIMULATION

Nasal mucosa of the inferior turbinate were obtained from patients with allergic rhinitis (AR) when they underwent nasal surgery, as described previously.[9,13] Nasal specimens were cultured in 10 cm dishes containing medium (RPMI 1640; Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% heat-inactivated FCS (Gibco, Grand Island, NY, USA), 0.29 mg/ml glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin, at 37℃ in 5% CO_2 and humidified air. Nasal fragments were removed and the first passage was performed. After 3-4 weeks, nasal-mucosa-derived fibroblast cell lines were established. The cells were used at passage numbers 3-5. Using cytokeratin and vimentin markers, epithelial cells were confirmed not to be contaminated by immunohistochemical examination. The cells were then placed in a 24-well flat-bottomed tissue culture plate at an initial density of 1×10^5 cells/well for chemokine production or a 10 cm dish for Western blot and RT-PCR.

CYTOKINE AND CHEMOKINE ASSAY

The cells were cultured in the presence of cytokines (IL-4, IFN-γ, IL-19) for appropriate periods; then culture supernatants were harvested. Amounts of chemokines in the cell culture supernatant were measured using commercially available ELISA kits. Measurements were performed according to the manufacturer's directions. All samples were assayed in duplicate.

RT-PCR ANALYSIS

Total RNA was extracted using a total RNA isolation NucleoSpin™ RNA II Kit (Macherey-Nagel, Duren, Germany). The reverse transcription reaction was performed using Taqman RT Reagents (Applied Biosystems Japan, Tokyo, Japan) with random hexamer primers. Using 2 μg of total RNA, first-strand cDNA was synthesized using a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Then 1 μl of the resulting first-strand cDNA was used for each PCR.[16] The following primers for human IL-20R1 and IL-20R2 were used in the reactions: IL-20R1, 5'-TCAAACAGAACGTGGTCCCA GTG-3' (nucleotides 1001-23) and 5'-TCCGAGATATT GAGGGTGATAAAG-3' (nucleotides 1369-92), IL-20R 2, 5'-GCTGGTGCTCACTCACTGAAGGT-3' (nucleotides 509-31), and 5'-TCTGTCTGGCTGAAGGCGCT GTA-3' (nucleotides 892-914). The reaction mixture in a final volume of 25 μl consisted of 1 × Taq DNA polymerase buffer, 0.2 mM dNTP, 1.5 mM MgCl2, 0.5 μM of each primer, and 2.5 U of Taq DNA polymerase (Amersham Pharmacia Biotech Inc.) according to the instructions for the Taq DNA polymerase. Preamplification denaturation was performed at 94℃ for 5 min and amplification was conducted for 35 cycles. The respective cycles were 94℃ for 1 min, 65℃ for 1 min, and 72℃ for 1 min. The final extension step was performed for 7 min at 72°C. Samples (10 μl) of the PCR products were analyzed on 2% agarose gel in 1 × Tris-acetate-EDTA (TAE) buffer; bands were visualized using ethidium bromide staining.

REAL TIME PCR

Total RNA was extracted and the reverse transcription reaction was performed as described above. The amplifications of SOCS-1, SOCS-3, SOCS-5, and β2-microlobulin-cDNA were performed in a MicroAmp optical 96-well reaction plate (Applied Biosystems Japan). All TaqMan probe/primer combinations used for this study were TaqMan Gene Expression Assay products purchased from Applied Biosystems. Because it is convenient to assay and because it is highly expressed, β2-microglobulin was chosen as the reference housekeeping gene. Furthermore, to select the housekeeping gene, we evaluated it using a TaqMan Human Endogenous Control Plate, which is

most suitable. TaqMan PCR was performed in a 20 μl volume using TaqMan Universal PCR master mix (Applied Biosystems Japan). The reaction was performed using a sequence detection system (ABI Prism 7000; Applied Biosystems Japan). Reaction mixtures were pre-incubated for 2 min at 50℃. The PCR program was 10 min of Taq Gold activation at 95℃, followed by 40 cycles of 15 s at 95℃ and 1 min at 60℃ (maximum ramping speed between temperatures). Human cDNA equivalent to 50 ng of total RNA from each sample was assayed in each tube.

The threshold cycle number (Ct) was determined using sequence detector software (ver. 1.1: Applied Biosystems Japan) and transformed using comparative Ct methods, as described by the manufacturer, with β2-microglobulin as the calibrator gene.

RNAi AGAINST IL-20R2 AND SOCS-1

Human IL-20R2 siRNA (5'-GAUGGCUUCCACCUGG UUA TT-3) and a nonspecific scrambled siRNA (5'-A GUUCUGGUCGCCGAUCUA TT-3') were purchased from Takara Bio Inc. (Otsu, Japan). Human SOCS-1 siRNA (5'-GGGUCUCUGGCUUUAUUUU TT-3') was purchased from Applied Biosystems Japan. Nasal fibroblast cells were transiently transfected with 1.3 μmol/L of specific siRNA targeting human IL-20R2, SOCS-1, or nonspecific siRNA for 2 hr using a Trans IT-TKO (Mirus Bio LLC, Madison, WI, USA) system according to the manufacturer's instructions. The medium was replaced with conditioning medium for an additional 24 hr.

WESTERN BLOT ANALYSIS

Human nasal fibroblasts were washed twice with ice-cold PBS and collected by scraping, then centrifuged and pelleted at 4℃. The nasal fibroblasts were homogenized in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 1 mM PMSF, 0.5 mM EDTA, 0.6 μM leupeptin, 2 μM pepstatin A, and 1 mM PMSF] by pipetting and sonication. Protein concentrations were measured in all experiments using the BioRad Protein Assay Kit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Lysates were centrifuged at 10000 rpm for 10 min at 4℃; the supernatants were used for immunoblotting. The supernatants were added to a twofold volume of sample buffer [95% Laemmli sample buffer (Bio-Rad Laboratories Inc.) and 5% 2-mercaptoethanol]. After heating at 95℃ for 5 min, the samples were electrophoresed. Proteins were transferred electrophoretically onto polyvinylidene fluoride (PVDF) membranes (Amersham Hybond-P, GE Healthcare, Tokyo, Japan). The blotted membranes were then rinsed with 5% non-fat dry milk diluted in PBS containing 0.1% Tween 20 for 60 min at room temperature. They were incubated overnight at 4℃ with the anti-STAT6 and anti-phospho-STAT6 polyclonal antibody (Ab) (1/1000 in 5% BSA in TBST) (Cell Signaling Technologies, St. Louis, MO, USA). After being washed, the membranes were treated with HRP-conjugated anti-mouse immunoglobulin (Ig) Ab or HRP anti-rabbit Ig Ab (Dako, Carpinteria, CA, USA) for 60 min at room temperature. The blot was washed again; then it was developed using the ECL plus Western blot detection reagents (Amersham Pharmacia Biotech Inc.).

IMMUNOHISTOCHEMICAL STAINING

Nasal specimens from patients with AR were obtained by turbinate surgery. Paraffin-embedded blocks were sliced into 4-μm-thick sections. The sections were deparaffinized with xylene, dehydrated in ethanol, and put into methanol containing 0.3% H_2O_2 for 15 min to block endogenous peroxidase activity. The sections were then incubated with normal bovine serum for 15 min at room temperature and treated overnight at 4℃ with anti-IL-19 monoclonal antibody (Santa Cruz Biotec Inc., CA, USA). The sections were then washed with PBS and incubated with a cocktail of peroxidase-labeled polymer conjugated to goat anti-mouse IgG antibody and goat anti-rabbit IgG antibody at room temperature for 1 h. Finally, the sections were incubated in PBS containing 0.03% DAB and 0.01% H_2O_2. All slides were lightly counterstained with Mayer's hematoxylin.

DATA AND STATISTICAL ANALYSES

Data in the text and figure legends are expressed as the mean ± SEM of observations. Statistical analyses were Wilcoxon signed-rank tests used to assess the difference in chemokine production levels. Computers with Microsoft Windows OS with software (Statview; Abacus Concepts Inc., Berkeley, CA, USA) were used for all statistical analyses.

RESULTS

HUMAN NASAL FIBROBLASTS EXPRESS IL-20 R1 AND IL-20R2

We first set out to determine the expression of IL-20R1 and IL-20R2 because IL-19 binds two distinct receptor complexes consisting of the IL-20R1 and IL-20R2 chains.[17-19] The expression of mRNA components for IL-20R1 (392 bp) and IL-20R2 (406 bp) were determined in all nasal fibroblasts (n = 6) using RT-PCR (Fig. 1a). These bands were fully sequenced and identified as IL-20R1 and IL-20R2 (data not shown).

NASAL EPITHELIA EXPRESS IL-19

Ten formalin-fixed paraffin-embedded nasal mucosa specimens were stained using ABC methods with anti-human IL-19 monoclonal antibody. The cytoplasm and plasma membranes of epithelial cells were positively stained (Fig. 1b), as were infiltrated cells in the lamina propria, indicating the possible production of IL-19 by inflammatory cells.

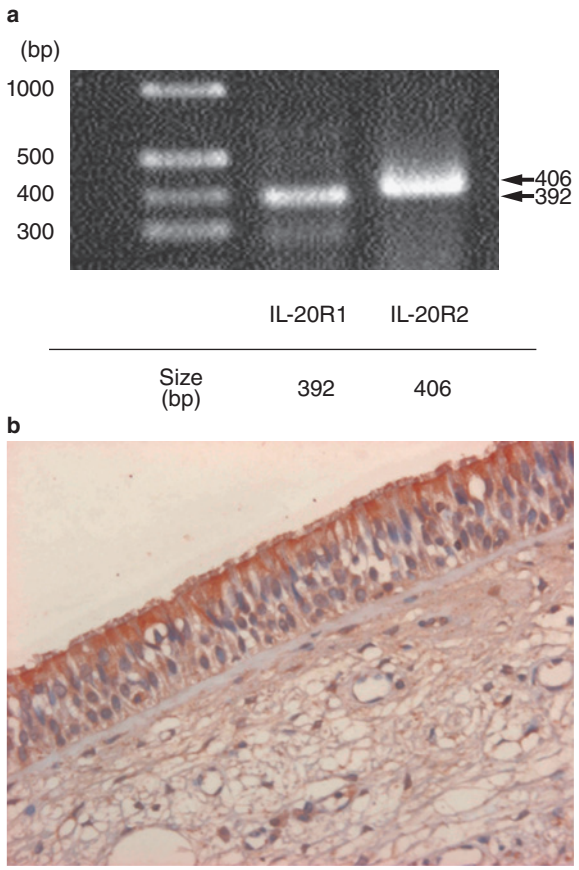

Fig. 1 (**a**) Expression of components of IL-19 receptors in human nasal fibroblast was determined using RT-PCR. IL-19 binds to IL-20R1 and IL-20R2. All nasal fibroblasts (n = 6) express both IL-20R1 and IL-20R2. (**b**) Immunohistochemical staining of IL-19 in nasal mucosa showed epithelial cells and infiltrated cells positively.

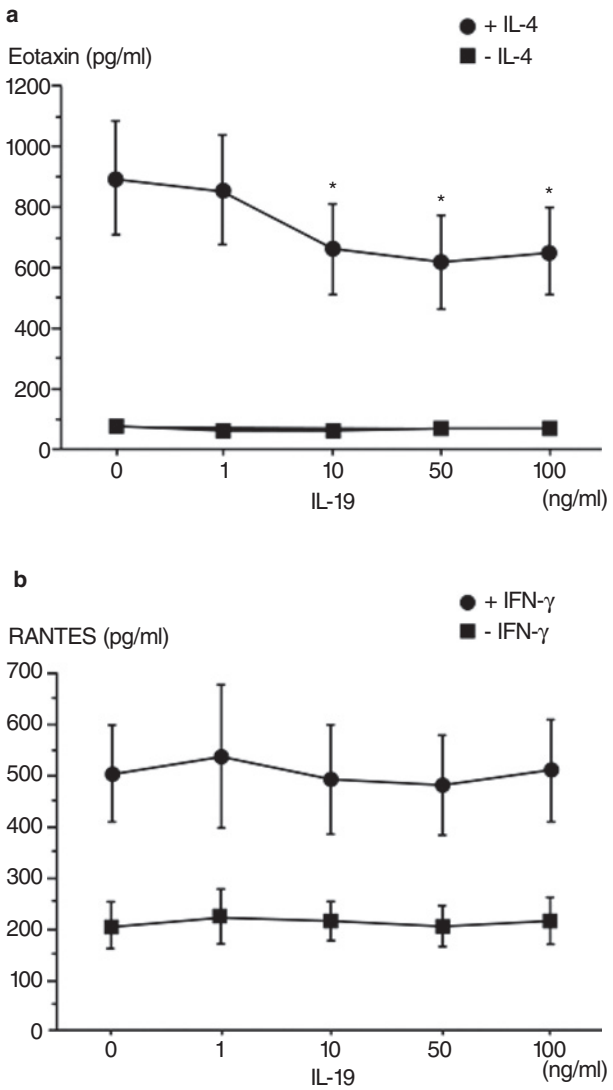

Fig. 2 IL-4 induced eotaxin, and IFN-γ induced RANTES by human nasal fibroblasts. Nasal fibroblast cells were pretreated with IL-19 (0, 1, 10, 50, 100 ng/ml) for 24 hr and stimulated with IL-4 (10 ng/ml) (**a**) or IFN-γ (30 ng/ml) (**b**) for 24 hr. (**a**) IL-19 inhibited IL-4-induced eotaxin production (n = 6). * $p < 0.05$ compared to eotaxin value of the supernatant in the presence of IL-4 without IL-19. (**b**) IL-19 did not act on IFN-γ-induced RANTES production (n = 6).

IL-19 SUPPRESSED EOTAXIN PRODUCTION BY IL-4 IN HUMAN NASAL FIBROBLASTS

Nasal fibroblasts can release various chemokines. Actually, IL-4, a representative Th2 cytokine, induced eotaxin in human nasal fibroblasts[13] although IFN-γ, Th1 cytokine induced RANTES.[20] Simultaneous stimulation with IL-4 (10 ng/ml) and IL-19 (50 ng/ml) for 24 hr induced equal amounts of eotaxin production to that by IL-4 alone from nasal fibroblasts (IL-19 + IL-4, 870 ± 205 pg/ml vs. IL-4, 822 ± 212 pg/ml, mean ± SE). Eotaxin production by simultaneous stimulation with IL-4 and IL-19 for 48 hr was not different from that obtained with IL-4 alone (data not shown).

Nasal fibroblasts were pretreated with each concentration of IL-19 for 24 hr and were then stimulated with IL-4 (10 ng/ml) for 24 hr. Significantly enhanced inhibition of eotaxin production was observed with 10 ng/ml of IL-19 (IL-19 + IL-4, 618 ± 153 pg/ml vs. IL-4, 896 ± 191 pg/ml, $p < 0.05$) (Fig. 2a). The suppression of eotaxin production reached a plateau from stimulation with 10 ng/ml of IL-19. The time course of IL-19 effects was determined. Inhibition of eotaxin production by IL-19 reached a plateau at 24 hr of pretreatment (data not shown). No difference in cell viability was found between IL-4 alone and IL-4 + IL-19. The nasal fibroblast shape was not altered by treatment with IL-19 (data not shown). We inferred that the optimal pretreatment time and concentration were, respectively, 24 hr and 10 ng/ml of IL-19. Pretreatment of IL-19 was critical for the inhibition of eotaxin production in nasal fibroblasts.

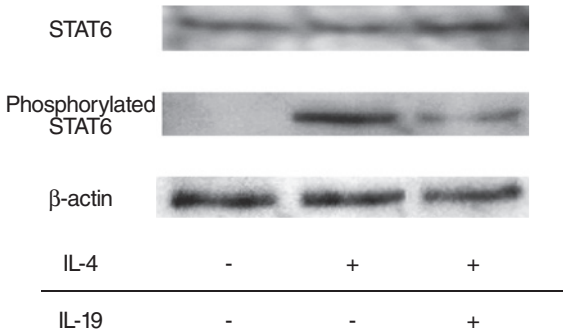

Fig. 3 IL-19 suppresses IL-4-induced STAT6 phosphorylation. Nasal fibroblasts were cultured in medium for 26 hr (lane 1), or with IL-4 (10 ng/ml) for 2 hr after incubation in medium for 24 hr (lane 2), or with IL-4 (10 ng/ml) for 2 hr after pretreatment of IL-19 (10 ng/ml) for 24 hr (lane 3). Samples were blotted with STAT6 Ab and phosphorylated STAT6 Ab.

The TNF-α enhanced eotaxin production by IL-4 in fibroblasts. The addition of TNF-α (50 ng/ml) to IL-4 enhanced eotaxin production (2537 ± 503 pg/ml) three-fold. Pretreatment of IL-19 (10 ng/ml) also inhibited TNF-α + IL-4-induced eotaxin production (1839 ± 330 pg/ml, $p < 0.05$ compared to TNF-α + IL-4). The inhibition rate of eotaxin production by IL-19 + TNF-α + IL-4 was not different from that by IL-19 + IL-4 (31% vs. 28%).

Nasal fibroblast released RANTES by IFN-γ (30 ng/ml) stimulation. Pretreatment of IL-19 (10 ng/ml) had no effect on RANTES production in nasal fibroblasts (IL-19 + IFN-γ, 491 ± 106 pg/ml vs. IFN-γ, 505 ± 95 pg/ml) (Fig. 2b). Although the concentration of IL-19 was changed, no effect of IL-19 on RANTES production was found. Simultaneous stimulation with IFN-γ and IL-19 had no effect on RANTES production (data not shown).

IL-19 SUPPRESSED IL-4-INDUCED STAT6 PHOSPHORYLATION

Results of previous studies show that the signal transducer and activator of transcription 6 (STAT6) was necessary for IL-4-induced eotaxin production.[21] We investigated the effect of IL-19 on tyrosine phosphorylation of STAT6. No phosphorylation of STAT6 was observed in unstimulated fibroblasts. When nasal fibroblasts were stimulated with IL-4 for 2 hr, tyrosine phosphorylation of STAT6 was induced (Fig. 3). However, pretreatment with IL-19 for 24 hr before stimulation with IL-4 reduced the amount of phosphorylated STAT6 in nasal fibroblasts.

IL-19 INDUCED SOCS-1 IN NASAL FIBROBLASTS

We tested next whether IL-19 might regulate early receptor-mediated events: expression of the SOCS regulatory proteins. The SOCS proteins–SOCS-1,[22] SOCS-3,[23] and SOCS-5[24] reportedly inhibit the IL-4/STAT6 signal transduction pathway. For that reason, we used real-time PCR to investigate whether expression of SOCS-1, SOCS-3, and SOCS-5 were induced by pretreatment with IL-19 for 24 hr in nasal fibroblasts. Treatment with IL-19 (10 ng/ml) for at least 2 hr significantly up-regulated the mRNA for SOCS-1 (3.0-fold compared to unstimulated cells, $p < 0.05$; Fig. 4a). The high level of mRNA for SOCS-1 continued to 24 hr after IL-19 stimulation. However, respective mRNA expressions of SOCS-3 and SOCS-5 were not enhanced by IL-19 treatment (Fig. 4b, c).

IL-19 HAD NO ENHANCEMENT OF SOCS-1 WHEN IL-20R2 WAS KNOCKED DOWN BY RNAi

To confirm that IL-19 enhanced SOCS-1 mRNA, IL-20R2 was changed artificially using RNAi method. Nasal fibroblasts were treated with RNAi according to the manufacturer's instructions. For nasal fibroblasts, the RNAi knockdown caused 49.3% reduction of IL-20R2 mRNA using real-time PCR.

Pretreatment with IL-19 enhanced the expression of SOCS-1 mRNA in the control siRNA-transfected cells (Fig. 5). However, the transfection of IL-20R2 siRNA abolished IL-19-enhanced SOCS-1 mRNA expression (Fig. 5, lane 4). No significant difference in cell viability was found among controlled fibroblasts and transfected fibroblasts with control RNAi or IL-20 R2 RNAi (data not shown). Eotaxin production was reversely correlated with SOCS-1 mRNA expression. Transfection of IL-20R2 siRNA to nasal fibroblasts increased IL-4-induced eotaxin production in the presence of IL-19 compared to fibroblasts treated with control siRNA (IL-20R2 RNAi, 476.2.1 ± 75.5 pg/ml vs. control RNAi, 342.0 ± 70.2 pg/ml, $p < 0.05$).

IL-19 SHOWED NO SUPPRESSION OF IL-4-INDUCED EOTAXIN PRODUCTION WHEN SOCS-1 WAS KNOCKED DOWN BY RNAi

Nasal fibroblasts were transfected with SOCS-1 siRNA to examine direct effects of SOCS-1 on suppression of IL-4-induced eotaxin production by IL-19. For nasal fibroblasts, RNAi knockdown caused 41.2% reduction of SOCS-1 mRNA by real time PCR. The transfection of SOCS-1 siRNA showed no enhancement of IL-4-induced eotaxin compared to that of the control siRNA in the absence of IL-19 (SOCS-1 RNAi, 564.0 ± 68.6 pg/ml vs. control RNAi, 523.4 ± 60.4 pg/ml, Fig. 6). Pretreatment with IL-19 reduced IL-4-induced eotaxin in the control siRNA transfected cells (381.0 ± 30.8 pg/ml, $p < 0.05$ compared to that in the absence of IL-19). However, pretreatment with IL-19 in the SOCS-1 siRNA transfected cells showed no suppression of IL-4-induced eotaxin production (540.6 ± 58.4 pg/ml). Cell viability and form in SOCS-1 siRNA transfected cells were not different from those in control siRNA transfected cells (data not shown).

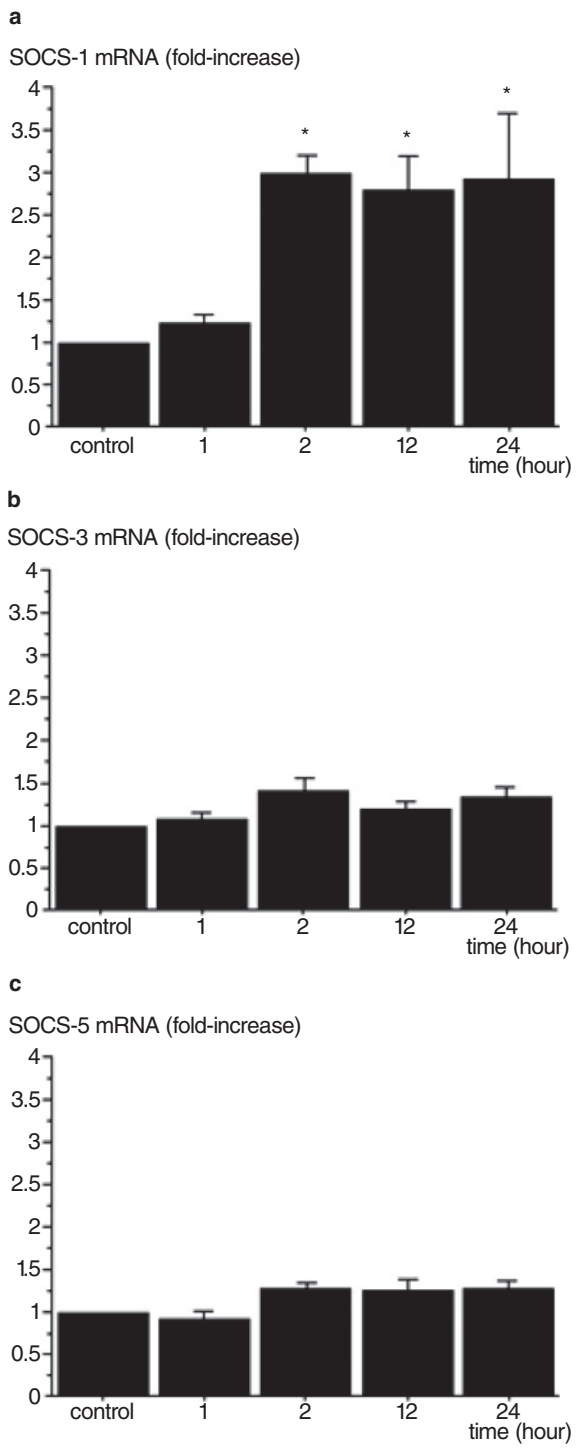

Fig. 4 IL-19 induced SOCS-1 (**a**), but not SOCS-3 (**b**) or SOCS-5 (**c**) by nasal fibroblasts. Nasal fibroblasts were cultured with or without IL-19 (10 ng/ml) each time. Respective expressions of SOCS-1, SOCS-3, and SOCS-5 in nasal fibroblasts were assayed using real-time PCR. Reactions were performed in three wells. The results are expressed relative to expression levels of β2-microglobulin. Data are presented as the mean ± SEM (n = 9). * $p < 0.05$ compared to control.

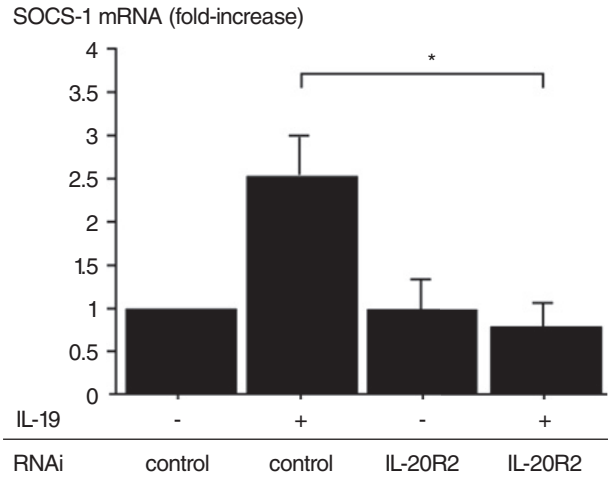

Fig. 5 Knockdown of IL-20R2 by RNAi abolished IL-19-induced SOCS-1 expression. Nasal fibroblasts were transfected by IL-20R2 siRNA or control siRNA. Then SOCS-1 mRNA was assayed using real-time PCR. Reactions were performed in three wells. The results are expressed relative to expression levels of β2-microglobulin. Data are presented as the mean ± SEM (n = 5). Knockdown of IL-20R2 by RNAi reduced SOCS-1 mRNA expression to the level of the control level. * $p < 0.05$.

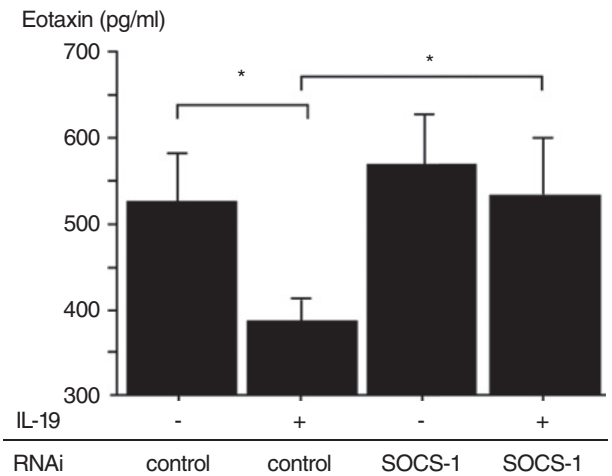

Fig. 6 Knockdown of SOCS-1 by RNAi abolished the suppression of IL-4-induced eotaxin production by IL-19. Nasal fibroblasts were transfected with SOCS-1 siRNA or control siRNA. Concentration of eotaxin in the supernatant of the culture system was assayed using ELISA. * $p < 0.05$.

DISCUSSION

In this study, we demonstrated that IL-19 inhibited IL-4-induced eotaxin production from human nasal fibroblasts, but not IFN-γ-induced RANTES. In fact, IL-19 induced SOCS-1 signaling, and the SOCS-1 signal

suppressed IL-4-induced tyrosine phosphorylation of STAT6 in nasal fibroblasts. The SOCS-3 and SOCS-5 mRNA were not induced by IL-19. These results suggest that IL-19 played a suppressive role of eosinophil-induced inflammation via suppression of eotaxin production in nasal fibroblasts of patients with AR.

The functions of many interleukins are elaborated by activation of intracellular signaling cascades involving the SOCS and STAT family of signaling proteins. Six SOCS family members exert their inhibitory effects by binding to tyrosine phosphorylated residues on signaling intermediates, protein kinases, and receptor chains, resulting in the attenuation of signaling. Then SOCS-1 and SOCS-3 inhibited IL-4-induced secretion of eotaxin in HEK293 cells.[25] In fact, IL-4-induced STAT6 activation was inhibited profoundly by 24 hr pretreatment with interferon (IFN)-γ in human primary airway epithelial cell cultures because IFN-γ pretreatment induced both SOCS-1 and SOCS-3.[26] Another group demonstrated that IFN-γ-induced SOCS-1 regulated STAT6-dependent eotaxin production triggered by IL-4 and TNF-α in mouse embryonic fibroblast from SOCS-1 knockout mice.[27] Our data, showing that IL-19-induced SOCS-1 inhibited eotaxin production from human nasal fibroblast via suppression of IL-4-induced STAT6, are consistent with evidence reported in the literature.

As the IL-19-inducible inhibitory signal transduction for inflammation, SOCS-5 was reported in vascular smooth muscle cells.[14] However, nasal fibroblasts did not increase SOCS-5 signaling by IL-19. Consequently, the signal pathway of IL-19 is inferred to be dependent upon the cell type.

Actually, IL-19 exerts its effect via the type I IL-20 receptor complex consisting of IL-20R1 and IL-20R2. The IL-19 first binds to IL-20R2,[18,19] which leads us to knockdown of IL-20R2 by RNAi in nasal fibroblasts. Recently, Wahl *et al*. reported that IL-20R2 knockout mice were sensitive to the contact allergen. The IL-20 R2 signaling directly regulated CD4 and CD8 T cell response *in vitro* and *in vivo*.[28] Our study showed that regulation of IL-20R2 using the RNAi method abolished the effect of IL-19 on IL-4-induced eotaxin production. These facts suggest that IL-20R2 might be critical for the function of IL-19.

A survey of the distribution of IL-19 protein was performed using healthy human tissues of 28 types with tissue microarray and immunohistochemical staining. The major cell types that stained positive for IL-19 were epithelial cells, endothelial cells, and macrophages.[29] Results showed IL-19 expression to a great degree in airway epithelial cells from asthmatic patients, synergistically activated by IL-13 and IL-17A.[15] We also found IL-19 expression in nasal epithelial cells and infiltrated cells from AR patients. Proinflammatory cytokine IL-1β induced a great increase of IL-19 expression (more than 1000-fold increase) in keratinocytes.[30] The inflammatory response that occurs during cardiopulmonary bypass has often been described as a systemic inflammatory response syndrome resembling sepsis. The level of IL-19 in serum was elevated in cardiac surgery with cardiopulmonary bypass and its rise occurred concomitantly with the induction of IL-10, IL-6, and TNF-α.[31] Endotoxic shock is a systemic inflammatory response to severe bacterial infections. Serum levels of IL-19 are reportedly higher in patients with endotoxic shock than in healthy volunteers.[32] Uremic patients on hemodialysis are in a chronic state of inflammation. Expression of IL-19 correlated with proinflammatory cytokines and Th2 cytokine production in uremic patients on hemodialysis.[33] Psoriasis is a chronic inflammatory skin disease, and in fact, IL-19 expression *in vivo* is elevated in psoriatic skin.[34] In the diseased skin of atopic dermatitis, IL-19 is also highly expressed.[30] These results suggest that IL-19 and several proinflammatory cytokines contribute interactively to inflammatory responses. However, the valuable function of IL-19 remains unclear.

Apoptosis is important as a mechanism of inflammation. However, the regulating mechanism of IL-19 on inflammation has remained controversial. Liao *et al*. demonstrated that treatment of mouse monocyte with IL-19 induced production of IL-6 and TNF-α. Actually, IL-19 also induced mouse monocyte apoptosis and the production of reactive oxygen species.[35] Compared to normal fibroblasts, no difference of trypan blue or PI staining was found in nasal fibroblasts by pretreatment with IL-19 (data not shown). These results suggest that apoptosis is not induced by IL-19 treatment in human nasal fibroblasts.

Anti-proliferative effects of IL-19 on a cancer cell line have been reported.[19] Tian *et al*. also demonstrated a dose-dependent, anti-proliferative effect of IL-19 on primary human coronary artery vascular smooth muscle cells (VSMCs).[14] In contrast, it has been reported that IL-19 induced proliferation of oral cancer cell lines.[29] Results of the present study showed no significant difference of absorbance values by MTT assay evaluating cell growth in nasal fibroblast between pretreatment with or without IL-19 (data not shown). This result demonstrated that the reduction of eotaxin production by pretreatment with IL-19 was not associated with cell growth by nasal fibroblasts.

Fibroblasts established from rheumatoid synovium produce IL-19 and constitutively express both IL-20R1 and IL-20R2. Furthermore, IL-19 induced STAT3 activation and increased IL-6 production by rheumatoid synovium; IL-19 significantly reduced apoptosis of rheumatoid synovium induced by serum starvation.[36] However, nasal fibroblasts were not confirmed by RT-PCR to express IL-19 mRNA (data not shown). Consequently, nasal fibroblasts differ from the fibroblasts of rheumatoid synovium.

Nasal mucosa play a crucial role as the first line of host defense mechanism against invading pathogens. The attack of invading pathogens induces toll-like receptor (TLR) signals in nasal mucosa. Nasal fibroblast and epithelial cells express TLR1, 2, 3, 4, 5, 6, and 9.[13] The TLR signals induce proinflammatory cytokine and expand an allergic inflammation with Th2 cytokine. Viral and bacterial infections clinically produce nasal symptoms to a worse degree in patients with AR. In actuality, SOCS-1 plays an important role in quenching the activation of TLR signal-induced inflammation.[37] Our data imply to us that proinflammatory cytokine induces IL-19 from epithelial cells and that IL-19 affects nasal fibroblasts and suppresses allergic inflammation in an anti-inflammatory role. The regulatory cytokine IL-19 functions in an extremely complicated manner. Our data constitute valuable information that will support additional investigations of the biological function and clinical implications of IL-19 in humans.

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CONFLICT OF INTEREST

No potential conflict of interest was disclosed.

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