

Original article

Opposing roles of IL-17A and IL-25 in the regulation of TSLP production in human nasal epithelial cells

Background: The importance of IL-17A, IL-17F, and IL-25 in allergic rhinitis (AR), as well as their possible role in regulation on thymic stromal lymphopoietin (TSLP) production in nasal epithelial cells, is not well understood.

Objective: To determine the possible regulation of IL-17A, IL-17F, and IL-25 on TSLP production in the initiation of allergic responses.

Methods: The levels of IL-17A, IL-17F, IL-25, and TSLP in nasal lavages of patients with AR were measured using an enzyme-linked immunosorbent assay (ELISA) and compared with that in normal controls. Then, primary human nasal epithelial cells (HNECs) were stimulated with dsRNA (0–75 µg/ml), as well as IL-17A (100 ng/ml), IL-17F (100 ng/ml), and IL-25 (100 ng/ml). The mRNA expression of IL-17A, IL-17F, IL-25, TSLP, as well as the chemokines CCL20, IL-8, and eotaxin was analyzed using quantitative real-time PCR, and their protein levels in the supernatants of cultured HNECs were determined by ELISA.

Results: Both TSLP and IL-17 cytokines are significantly elevated in patients with AR. dsRNA was found to increase the production of IL-17F, IL-25, TSLP, CCL20, and IL-8 in HNECs. Furthermore, IL-25 significantly enhanced dsRNA-induced TSLP production in primary HNECs and was dominant to the inhibitory effect of IL-17A on TSLP regulation.

Conclusions: Our study provides the first evidence that both IL-17F and IL-25 can be induced by dsRNA in HNECs. Despite of the opposing effects of IL-17A and IL-25 on TSLP regulation in HNECs, IL-25 was dominant to IL-17A, providing a plausible explanation for the simultaneous upregulation of IL-17 cytokines and TSLP in patients with AR.

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Allergic diseases, including allergic rhinitis (AR) and asthma, are characterized by intense eosinophil infiltrates, mucus hypersecretion, airway remodeling, and airway hyperresponsiveness (AHR) (1–3). These pathognomonic features are mainly mediated by antigen-specific Th2 cells and their cytokines, such as IL-4, IL-5, and IL-13 (1–3). Recent studies have shown that the IL-17 cytokine family is involved in the pathogenesis of AR and asthma (4, 5). However, the relevant molecular mechanisms are not well understood.

The IL-17 cytokine family consists of six members, IL-17A (or IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also IL-25), and IL-17F, with shared homologies (6, 7). IL-17F shares the greatest similarity with IL-17A (55% identity), whereas, IL-25 is the most distant (17%). IL-17A is mainly produced by the Th17 cells, IL-17F is produced by both Th17 and epithelial cells, and IL-25 is

mainly released from the Th2 cells, epithelial, and mast cells, but not Th17 cells. The function of IL-17A/IL-17F (neutrophil-inducing cytokine) and IL-25 (Th2-associated cytokine) is completely different. Interestingly, all of these cytokines have been shown to be upregulated in allergic conditions such as asthma and are linked to the pathology of these diseases, regardless of their differences (4, 5).

IL-25 was considered a critical factor in regulating the initiation of innate and adaptive allergic responses (8). For example, it has been shown that mouse IL-25 is expressed by lung epithelial cells as a result of innate immune responses to allergens. Systemic administration of IL-25 protein or overexpression of IL-25 induced elevated Th2 cytokine and eotaxin production, eosinophilia, increased serum IgE, mucus hyperplasia, and other pathological changes in many tissues, and blocking IL-25 was reported to attenuate allergic responses in mouse

models (9). In addition, in a mouse model of asthma, IL-17A and IL-17F have been shown to induce neutrophilic airway inflammation (10). These findings indicate the importance of IL-17A and IL-17F in the induction of neutrophilic airway inflammation. Importantly, recent studies using murine asthma models have shown that IL-17 receptor-deficient (IL-17R2/2) mice exhibited reduced recruitment of neutrophils and eosinophils into the airways (11), resulting from impaired priming of Th2 responses. It has also been demonstrated that IL-17A2/2 mice exhibited reduced Th2 responses to antigen sensitization (12). The reduced Th2 responses in IL-17A2/2 or IL-17R2/2 mice may be explained by the recently described role of IL-17A in germinal center reactions (13). On the other hand, it has been demonstrated that the neutralization of IL-17A at the effector phase of airway inflammation enhanced eosinophilic inflammation (10, 11). Therefore, IL-17A seems to play a dual role in the regulation of eosinophilic airway inflammation.

The IL-17 cytokine family has been demonstrated to be associated with AR, but the pathogenic mechanism was unclear (4). Given the fact that neutrophilia is not implicated in the development of AR, additional targets of IL-17 cytokine family members may exist to regulate nasal allergic responses. To address this issue, we investigated the regulation of the IL-17 cytokine family on the expression of thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, in patients with AR and primarily cultured human nasal epithelial cells (HNECs). It is known that TSLP plays a determinant role in regulating the initiation and maintenance of allergic immune responses (14). Overexpression of TSLP in the airway epithelium of patients with AR and asthma has been shown to be correlated with the severity of these diseases (15, 16). The ability of TSLP to act as the initiating cytokine at the top of a chain of immunological events in an allergic cascade that leads to clinical manifestations has also been established (17). The effects of IL-17 cytokines on TSLP production are not well documented. In Angkasekwinai's (8) recent report, the immortalized mouse lung epithelial cell line, MLE12 cells, overexpressed TSLP and eotaxin, after treatment with IL-25 for 6 h. On the other hand, IL-17A was shown to inhibit the expression of TSLP in human primary keratinocytes, suggesting a suppressive effect on allergic responses (18). In this study, we found that IL-17A and IL-25, as well as TSLP, were upregulated in the nasal lavages of patients with AR. It likely to be contradictory, because IL-17A and IL-25 were previously shown to play different roles in regulating TSLP production in epithelial cells. To clarify the conflict, we investigated the combined effects of IL-17 cytokines on dsRNA-stimulated TSLP production in primary HNECs. Our findings provide a plausible explanation for TSLP regulation by IL-17 cytokines in HNECs and may be relevant to the design of possible AR treatment strategies.

Patients and methods

Patients

The study included 24 house dust mite (HDM)-sensitized patients with AR (15 male and 9 female patients; mean age, 36.5 years) with a history of moderate-to-severe persistent AR and 18 healthy control subjects (11 male and 7 female subjects; mean age, 31.1 years; Table 1). All subjects were healthy (except for AR) without any active disorders, including rhinosinusitis. Asthma was excluded in all patients with rhinitis. They were nonsmokers and free from upper respiratory tract infection for 4 weeks preceding the study. Skin prick tests and serum total and specific IgE detection were performed. AR diagnosis was based on their case history, complaints, nasal/endoscopic inspection, allergen skin prick tests, and specific IgE assays. The patients fit the standard criteria for AR, as stated in the ARIA guidelines (3). All subjects had no oral steroid intake or change in asthma medications during the 4 weeks prior to study recruitment. The study was approved by the local ethics committee and performed following the written informed consent of all participants.

Nasal lavages were collected by instillation of 3-ml sterile Phosphate Buffered Saline (PBS) into each nostril. The subject held it there for 5 s and then expelled it into a container. It was stored at -80°C for further analysis.

Cell culture, stimulation

Primary human nasal epithelial cells (HNECs) were collected randomly from the inferior turbinate of a normal control by nasal scrapping and dispersed into specific BEGM medium (Lonza, Walkersville, MD, USA) until passage. Cells, at passage 2–3, were grown in 24-well plates for use in experiments. At 80–90% confluence, HNECs were washed with PBS (37°C , pH 7.4), and fresh media (hydrocortisone-free) was added with polyriboinosinic:polyribocytidylic acid (poly I : C, 0–75 $\mu\text{g}/\text{ml}$; Sigma, St Louis, MO, USA) in the presence or absence of IL-17 cytokines (100 ng/ml for IL-17A, IL-17F, and IL-25; R&D Systems, Minneapolis, MN, USA). The cells were then incubated for 6 or 24 h. Next, cell pellets (6 h) and supernatants (24 h) were collected for quantitative real-time PCR and ELISA analyses, respectively.

Enzyme-linked immunosorbent assay

Cytokine and chemokine concentrations were measured using commercial ELISA kits (R&D Systems; detection limits: TSLP and IL-17A, 31.2 pg/ml; IL-17F and IL-25, 312.5 pg/ml; CCL20, 7.8 pg/ml; IL-8 31.2 pg/ml; and eotaxin, 15.6 pg/ml). For convenient analysis, all values below the detectable limit were considered to be zero.

Quantitative real-time PCR

Real-time PCR was performed as previously described (19). Briefly, total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Reverse transcription (RT) was performed, and the cDNA was synthesized from 2 μg of total RNA using an oligo (dT)18 primer and M-MLV reverse transcriptase (TAKARA, Syuzou, Shiga, Japan) for quantitative PCR. Expression of mRNA was determined using an ABI PRISM 7300 Detection System (Applied Biosystems, Foster City, CA, USA) and SYBR Premix TaqTM (TAKARA). The sequences of the primers are listed in Table 2. PRISM samples contained 1 $\times$ SYBR Green master mix, 1.5 μl of 5 μM primers, and 25 ng of synthesized cDNA

Table 1. Subject demographics

Groups	Control (<i>n</i> = 18)	AR (<i>n</i> = 24)	<i>P</i> -value
Sex (M : F)	11 : 7	15 : 9	
Age (years)	31.1 (21–44)	36.5 (20–51)	
Skin prick test-positive	0	24	
Asthma in history and present	0	2	
Smoking	0	0	
Duration of AR (years)	0	2–7	
Serum total IgE (KU/l)	9.2 (0–18.4)	144.2 (45.3–309.3)	<0.05
Serum specific IgE (KU/l)	<0.35	33.52 (3.9–88.4)	<0.05

Age, disease duration and IgE level are shown as median (range).
M, male; F, females; AR, allergic rhinitis.

in a 25-μl volume. Reactions were heated to 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, and annealing-extension at 60°C for 60 s. All PCR reactions were performed in duplicate. Melting curve analysis was used to control for amplification specificity. Expression of the target gene was expressed as a fold increase or decrease relative to the expression of β-actin. The mean value of the replicates for each sample was calculated and expressed as the cycle threshold (Ct). The amount of gene expression was then calculated as the difference (ΔCt) between the Ct value of target gene and the Ct value of β-actin. Fold changes in target gene mRNA were determined by $2^{-\Delta\Delta C_t}$.

Statistical analysis

Data are expressed as the mean ± SEM, except when stated otherwise. For nasal lavage examination, the levels of IL-17 cytokines and TSLP between patients with AR and controls were analyzed with the nonparametric Mann–Whitney *U*-test. The correlation between TSLP levels and IL-17 cytokines levels in patients with AR was assessed using a Spearman rank analysis. For cell culture results, data were obtained from at least three independent experiments and analyzed via one-way ANOVA and a paired *t*-test. A *P*-value of <0.05 was considered statistically significant.

Results

Levels of IL-17 cytokines and TSLP in nasal lavages of patients with AR

Previous studies have shown that IL-17A and TSLP were upregulated in patients with AR. To investigate the importance of IL-17 cytokines in AR, we examined three members of the IL-17 cytokine family (IL-17A, IL-17F, and IL-25) and TSLP in nasal lavages from AR subjects

in this study. A total of 24 patients with AR were included for nasal lavage collection and cytokines analysis (Table 1). We found the average levels of IL-17A, IL-17F, IL-25, and TSLP in nasal lavages were 48.1, 723.2, 1461.5, and 33.8 pg/ml, respectively. A significant elevation in both TSLP and IL-17 cytokines (IL-17A, IL-17F and IL-25) was observed in AR subjects, compared to the controls (*P* < 0.01, Fig. 1). Because IL-17A and IL-25 were demonstrated to modulate the expression of TSLP in human keratinocytes and mouse lung epithelial cells, respectively, we evaluated the relationship of IL-17 cytokines and TSLP production in nasal lavages of patients with AR. We found a statistically positive correlation between TSLP and IL-25 (*r* = 0.625, *P* < 0.05), while no correlation between TSLP and IL-17A or IL-17F (*r* = 0.112 and 0.430, respectively, *P* > 0.05) was observed.

Regulation of dsRNA on the expression of IL-17 cytokines and TSLP in primary HNECs

Based on the observation that IL-25 was associated with TSLP production in nasal lavages of patients with AR, we examined the effect of the TLR3 ligand dsRNA, a potent inducer of TSLP that mimics virus infection in airway epithelial cells (20), on expression of IL-17 cytokines and TSLP in primary HNECs. As a control, we first observed the spontaneous release of IL-17F and IL-25 from primary HNECs, while IL-17A and TSLP were almost absent, suggesting HNECs were a major source of IL-17F and IL-25. After stimulating with various concentrations of poly I : C (0–75 μg/ml), we found significant upregulation of IL-17F, IL-25, and TSLP mRNA and protein levels in primary HNECs, in a dose-dependent manner (Fig. 2, *P* < 0.05). No detection of IL-17A was observed in HNECs, confirming that epithelial cells were not the cellular source of IL-17A (7). Compared to high levels of IL-17F and IL-25, TSLP production was less sensitive to poly I : C treatment. For this reason, we used a higher concentration of poly I : C (50 μg/ml) to stimulate HNECs in the following experiments. As illustrated in Fig. 2, we found that IL-25 and IL-17F were significantly upregulated by poly I : C (mRNA: 4.9-fold for IL-25; 6.4-fold for IL-17F, *P* < 0.05; protein: for IL-25,

Table 2. Primers used for quantitative real-time PCR

Target gene	Forward (5′–3′)	Reverse (5′–3′)
Thymic stromal lymphopoietin (TSLP)	TATGAGTGGGACCAAAAGTACCG	GGGATTGAAGGTTAGGCTCTGG
IL-17A	CTGGAGGATAACACTGTGAGAGT	TGCTGAATGGCGACGGAGTTC
IL-17E	CCAGGTGGTGCATTCTTGG	TGGCTGTAGGTGTGGGTTC
IL-17F	AGCGCAACATGACAGTGAAG	GTGTAATCCAGGGGGAGGT
CCL20	CTGGCTGCTTTGATGTCAGT	CGTGTGAAGCCACAATAAA
IL-8	ATGACTTCCAAGCTGGCCGT	TCCTTGGCAAACTGCACCT
Eotaxin	CCCAACCACCTGCTGCTTAACCTG	TGGCTTTGGAGTTGGAGATTTGG
β-actin	TGCGTGACATTAAAGGAGAAG	GTCAGGCAGCTCGTAGCTCT

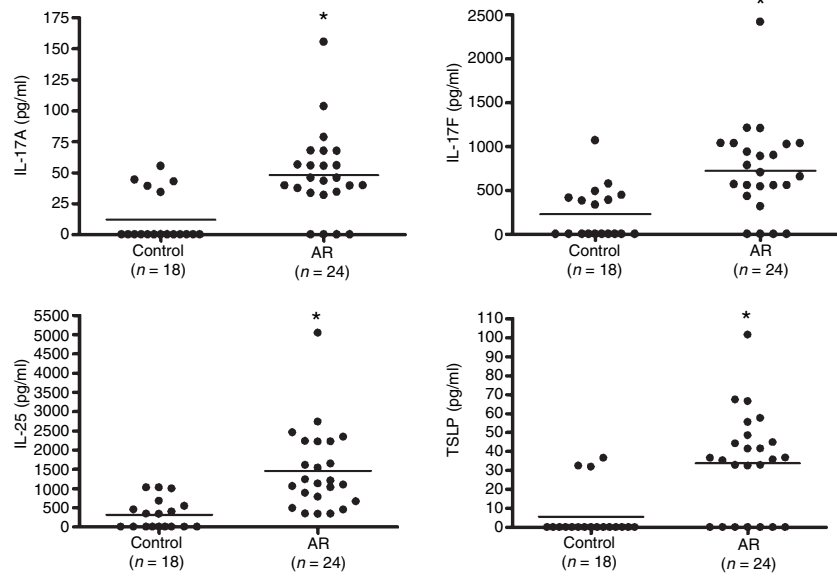


Figure 1. Levels of IL-17A, IL-17F, IL-25, and thymic stromal lymphopoietin (TSLP) in nasal lavages of patients with AR. Cytokine levels were measured in the nasal lavages of patients with AR ($n = 24$) and normal controls ($n = 18$) using ELISA. Data were analyzed using a nonparametric Mann–Whitney U -test. * $P < 0.01$, compared to the control group.

375.9 pg/ml vs 1024.6 pg/ml; for IL-17F, 442.3 pg/ml vs 1231.5 pg/ml), but not IL-17A (no detection). TSLP mRNA and protein levels were also significantly upregulated (for mRNA, 8.5-fold; for protein, no detection vs 47.5 pg/ml, $P < 0.05$), after poly I : C stimulation for 6 and 24 h; this was similar to previously reported results from cultures of lower respiratory epithelial cells (20).

Combined effects of IL-17 cytokines on dsRNA-stimulated TSLP production in primary HNECs

It was counterintuitive that both IL-17A and IL-25 were upregulated in patients with AR in this study, because IL-17A and IL-25 regulate TSLP production in epithelial cells differently (8, 18). To address the conflict, we investigated the combined effects of IL-17 cytokines on dsRNA-stimulated TSLP production in primary HNECs. As a control, there was no initial, spontaneous TSLP release. After stimulating with IL-17 cytokines and poly I : C, we found that IL-25 alone slightly enhanced TSLP mRNA production (for mRNA, 2.2-fold; for protein, no detection, Fig. 3), while IL-17A and IL-17F had no significant effects. On the other hand, TSLP mRNA and protein were significantly upregulated (mRNA: 8.5-fold; protein: no detection vs 47.5 pg/ml, $P < 0.05$) after poly I : C stimulation for 6 and 24 h.

In combination with poly I : C stimulation, IL-25 was found to significantly enhance dsRNA-induced mRNA and protein expression of TSLP in HNECs (mRNA: 15.4-fold; protein: 47.9 pg/ml vs 122.3 pg/ml, $P < 0.05$). Based on the previous result that dsRNA was able to

induce IL-25 production in HNECs, we concluded that dsRNA-induced TSLP production by a positive autocrine feedback loop, associated with IL-25 production. Therefore, we identified a novel, IL-25-dependent mechanism underlying the regulation of TSLP in the pathogenesis of allergic diseases. However, in contrast to the positive effect of IL-25, we found that IL-17A significantly inhibited dsRNA-induced mRNA and protein expression of TSLP in HNECs (mRNA: 35%; protein: 47.9 pg/ml vs no detection, $P < 0.05$), in contrast to IL-25 (Fig. 3). IL-17F also inhibited dsRNA-induced mRNA and protein expression of TSLP, but no significant difference was observed ($P > 0.05$). When IL-25, IL-17A, and poly I : C were administered simultaneously (IL-25 plus poly I : C, as well as IL-17A and IL-25 plus poly I : C), we found that the combinations significantly increased TSLP production in HNECs ($P < 0.05$). Although IL-17A was able to inhibit the synergistic effects of poly I : C plus IL-25 on TSLP production in HNECs, no significant difference was observed ($P > 0.05$). In other words, IL-25 was dominant to IL-17A, with respect to dsRNA-induced TSLP production in HNECs; this provided a reasonable explanation for the co-existence of antagonistic IL-17A and IL-25 in patients with AR.

Combined effects of IL-17 cytokines and dsRNA on chemokines production in primary HNECs

In addition, we examined the expression of chemokines (CCL20, IL-8, and eotaxin) in primary HNECs after treating with IL-17 cytokines and poly I : C. As illustrated in Fig. 4, we found that IL-17A and IL-17F, as

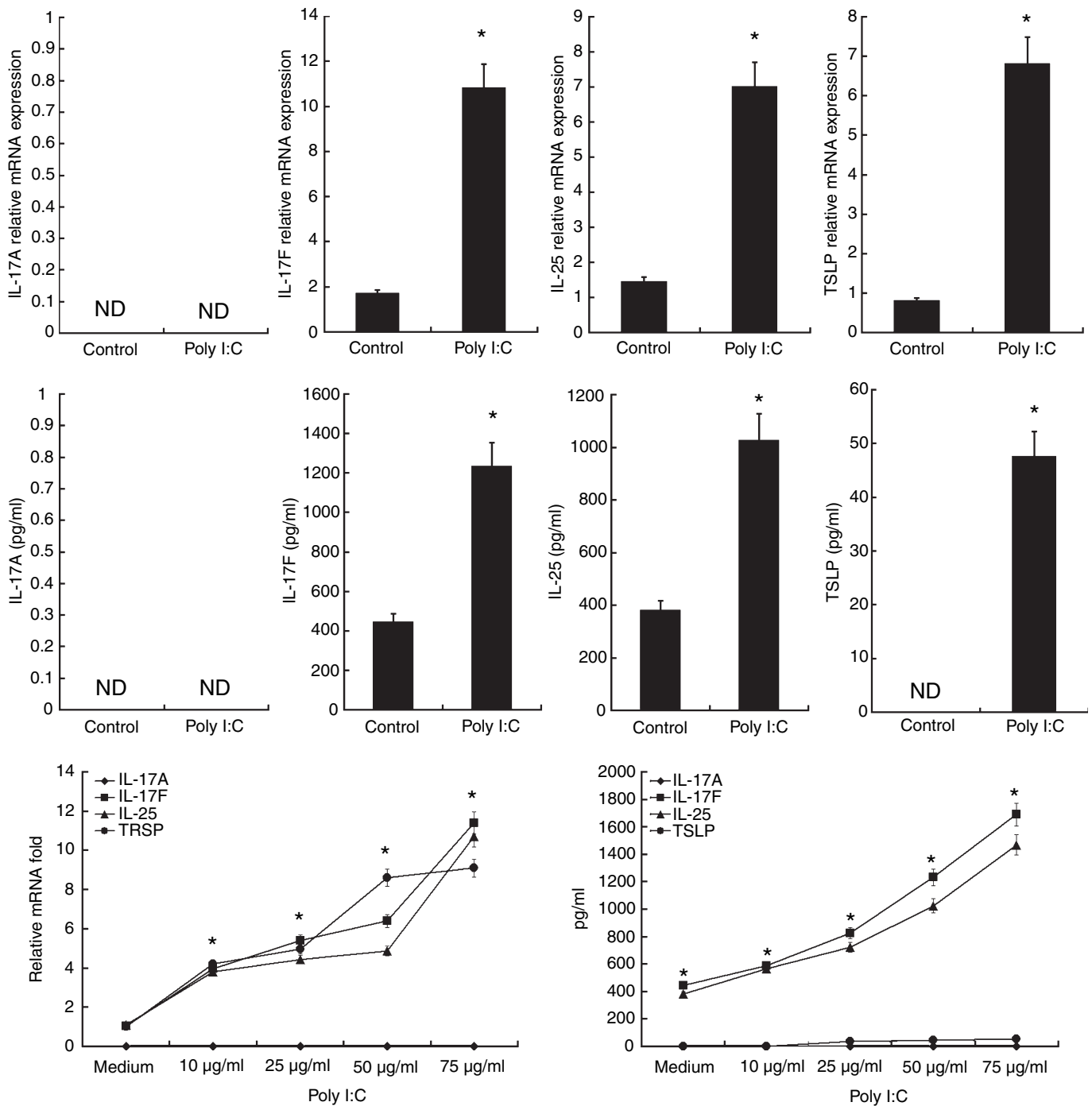


Figure 2. Effects of dsRNA on gene and protein expression of IL-17 cytokines and thymic stromal lymphopoietin (TSLP) production in primary HNECs, using quantitative PCR and ELISA. HNECs were treated with poly I : C (50 µg/ml) for 6 h (for mRNA detection) and 24 h (for protein assay). To evaluate the dose-response curve, poly I : C (0–75 µg/ml) was used for HNEC stimulation. Data were obtained from at least three independent experiments and analyzed by one-way ANOVA and paired *t*-test. **P* < 0.05, compared to the control group.

well as poly I : C, significantly enhanced CCL-20 and IL-8 gene and protein expression (*P* < 0.05), while IL-25 had no effect (*P* > 0.05). Our results confirmed that IL-17A and IL-17F, but not IL-25, played an important

role in dendritic cell and neutrophil migration (10). Moreover, both IL-17A and IL-17F were synergistic with poly I : C on CCL20 and IL-8 expression (*P* < 0.05), an effect that was not observed with IL-25 stimulation

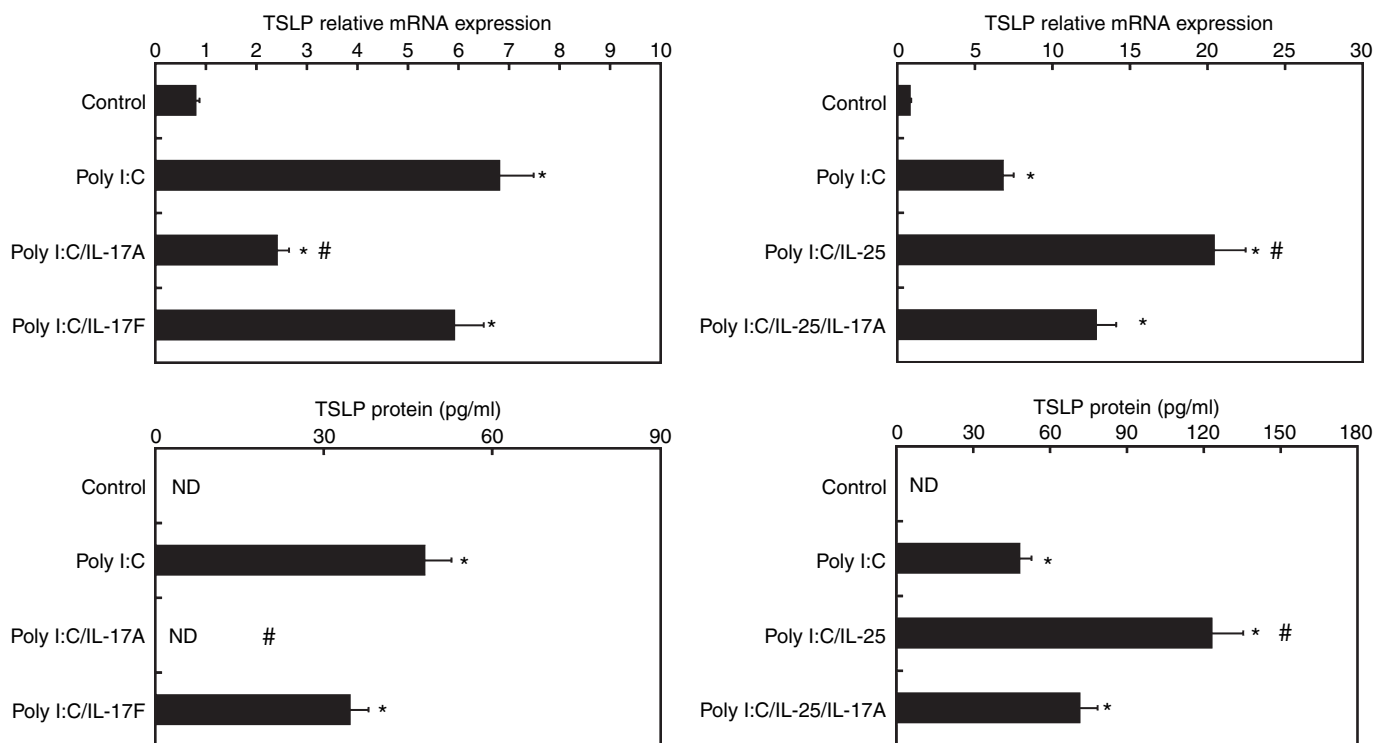


Figure 3. Combined effects of dsRNA and IL-17 cytokines on gene and protein expression of thymic stromal lymphopoietin (TSLP) in primary HNECs, using quantitative PCR and ELISA. HNECs were treated with poly I : C (50 µg/ml), IL-17A (100 ng/ml), IL-17F (100 ng/ml), and IL-25 (100 ng/ml) for 6 h (for mRNA detection) and 24 h (for protein assay). Data were obtained from at least three independent experiments and analyzed by one-way ANOVA and paired *t*-test. **P* < 0.05, compared to the control group; #*P* < 0.05, compared to the dsRNA-stimulated group.

(*P* > 0.05). However, IL-25 was found to significantly increase eotaxin production in primary HNECs (*P* < 0.05), confirming its role in eosinophil migration. Although poly I : C alone had no effect on eotaxin expression in HNECs, it showed a synergistic effect with IL-25 (*P* < 0.05). No significant suppression of IL-17A and IL-17F on IL-25-induced eotaxin production was observed, in contrast to TSLP regulation.

Discussion

In the present study, we showed that both the IL-17 cytokine family (IL-17A, IL-17F and IL-25) and TSLP were significantly upregulated in nasal lavages of patients with AR, compared to the normal controls. More importantly, we found that increased IL-25 was positively associated with TSLP production in patients with AR, suggesting possible regulation of TSLP production by IL-25 in the allergic response cascade. Because TSLP was recently established as a key factor in allergic inflammation, and overexpression of TSLP was associated with the severity of AR (14–16), the investigation of the regulation of TSLP production by the IL-17 cytokine family in HNECs was, therefore, of tremendous interest.

Despite the fact that both IL-17A/F and IL-25 were upregulated in patients with AR, their importance in the development of allergic responses is somewhat inconsistent. Most importantly, the functions of IL-17A/IL-17F (neutrophil-inducing cytokine) and IL-25 (Th2-associated cytokine) are completely different. IL-25 has been suggested as the key factor in allergic progression, Th2 polarization, and eosinophilia (8, 21). However, IL-17A was previously identified as a potent chemokine for neutrophilia associated with asthma exacerbation (10, 22), but IL-17A was also found to inhibit eosinophilic airway inflammation by suppressing the local allergic response (11); however, there is no evidence to suggest that neutrophils play an important role in AR (3). Therefore, this study is of importance, because it was performed in patients with AR that was different from asthma, to some extent. To date, information on IL-25 and IL-17F from patients with AR is lacking. Whether elevated IL-17A was responsible for AR is also unclear, although it was recently reported to be associated with AR severity. On the other hand, IL-17A was shown to inhibit the expression of TSLP in human primary keratinocytes (18), whereas TSLP was found to be upregulated in MLE12 cells after treatment with IL-25 (8), suggesting the different

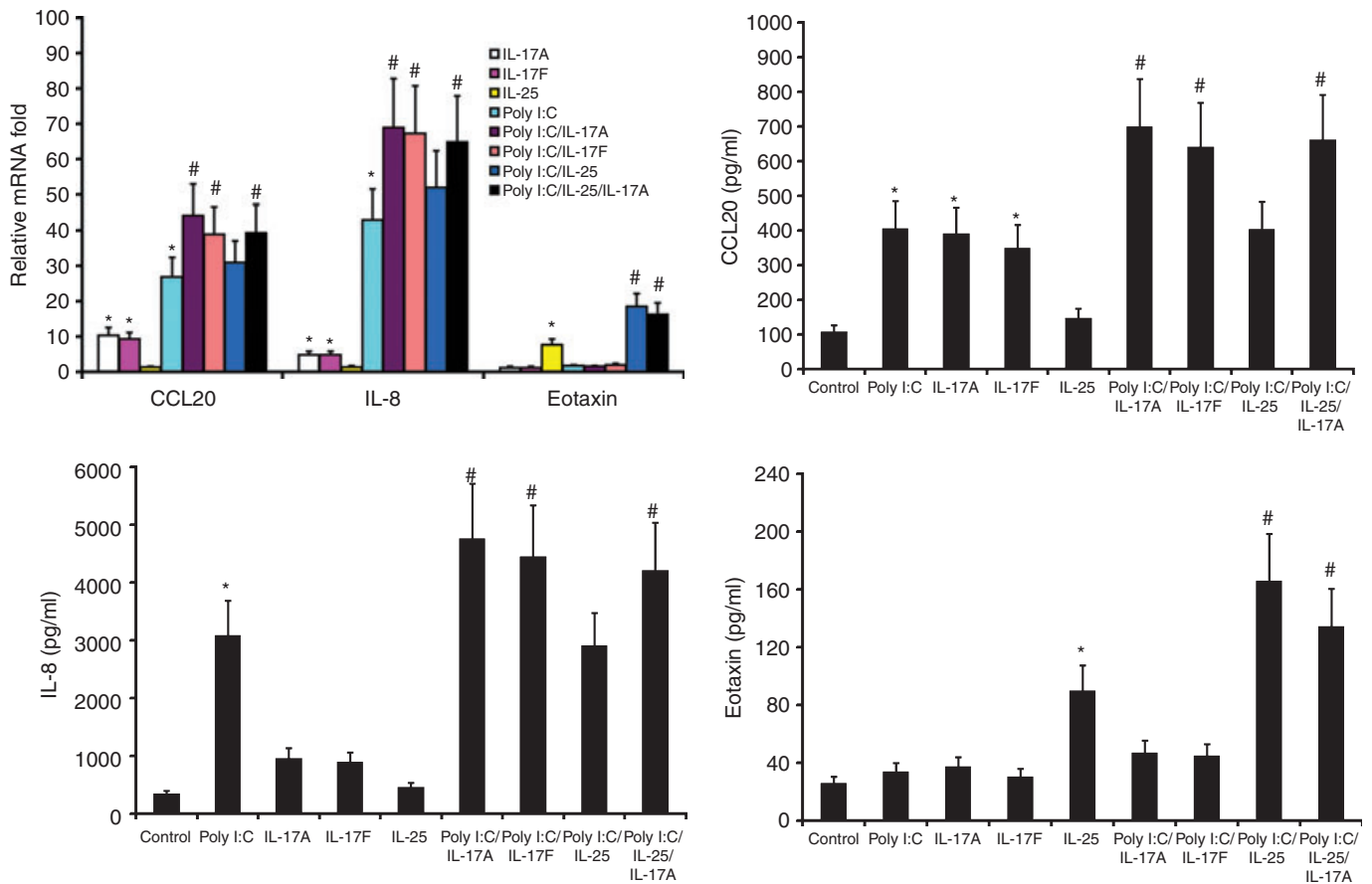


Figure 4. Combined effects of dsRNA and IL-17 cytokines on gene and protein expression of chemokines (CCL20, IL-8, and eotaxin) in primary HNECs, using quantitative PCR and ELISA. HNECs were treated poly I : C (50 μ g/ml), IL-17A (100 ng/ml), IL-17F (100 ng/ml), and IL-25 (100 ng/ml) for 6 h (for mRNA detection) and 24 h (for protein assay). Data were obtained from at least three independent experiments and analyzed using a one-way ANOVA and a paired *t*-test. **P* < 0.05, compared to the control group; #*P* < 0.05, compared to the dsRNA-stimulated group.

effects of IL-17A and IL-25 on TSLP production and allergic initiation.

In an attempt to explain the paradox in patients with AR, we established an *in vitro* primary HNEC culture system and evaluated the regulation of TSLP production after stimulation with members of the IL-17 cytokine family. We first investigated whether dsRNA could induce IL-17 cytokines and TSLP production in HNECs. dsRNA is known to be a substitute for viral RNA and viral replication. It is sensed by TLR3 and has been recently identified to be a potent inducer of TSLP, mimicking virus infection in the respiratory tract (20). The airway epithelial lining is the first immune barrier and was thought to be essential for linking innate and adaptive immunity. Therefore, the knowledge of whether airway epithelial cells produce IL-25 and the subsequent regulation of IL-25 is important for understanding allergic initiation and progression. We found that dsRNA induced significant upregulation of IL-17F, IL-25, and TSLP in primary HNECs, in a dose-dependent manner,

while no IL-17A was detected in stimulated HNECs. Our findings suggested that a different cellular source of IL-17 cytokines might be responsible for the apparent contradictory elevation in IL-17A and IL-25 levels in allergic responses. IL-17A was reported to be produced by Th17 cells only, while the cellular source of IL-25 has not been definitively identified (22, 23). In the mouse, IL-25 was first described as a Th2 cell-derived cytokine but was also expressed by mast cells (24, 25). Epithelial cells were recently identified as the cellular source of IL-25 in Angkasekwinai's study (8), and Zaph et al. reported that IL-25 production in intestinal epithelial cells is commensally dependent (26). In this study, we confirmed that HNECs were also the cellular source of IL-25 and provided evidence that IL-25 can be induced by a virus analog. However, the cell types responding to IL-25 and the mechanism by which IL-25 amplifies allergic immune responses remain elusive. The fact that HNECs produced IL-25 in response to dsRNA has enhanced our understanding of allergic inflammation. More interestingly, we

found that IL-25 alone slightly enhanced TSLP production in HNECs, and combined treatment with IL-25 and dsRNA significantly stimulated TSLP release from HNECs. These results suggest the existence of a positive autocrine feedback loop involving IL-25 and highlight the key role of IL-25 in nasal allergic initiation. Therefore, we concluded that HNECs are the critical cellular source of IL-25, and virus infection drives IL-25 production in HNECs, triggering the nasal allergic cascade.

Although IL-17A was increased in patients with AR and could be associated with AR severity (27), its importance was not completely understood (11, 28). It is known that IL-17A-mediated neutrophilia is not responsible for AR progression (3) that differs from asthma exacerbation. For this reason, it is imperative that we understand reasons for the co-existence of IL-17A and IL-25 in patients with AR. Here, we suspected that IL-17A could function as a negative regulator in initiation of AR, because it inhibited dsRNA-induced TSLP production in HNECs. Based on this supposition, the simultaneous elevation of IL-17A and IL-25 in patients with AR might not conflict with the apparently antagonistic effects of IL-17A and IL-25 on TSLP regulation in HNECs. To prove our hypothesis and further interpret the inconsistency between *in vivo* and *in vitro* data, we investigated the combined effects of IL-17A and IL-25 on dsRNA-induced TSLP production in HNECs. When IL-25, IL-17A, and dsRNA were administrated simultaneously, we found that the combined stimulus significantly increased TSLP production in HNECs, while IL-17A did not inhibit the synergistic effects of dsRNA plus IL-25 on TSLP production in HNECs. Regarding this, our results indicated that IL-25 was dominant to IL-17A. Thus, we provided a reasonable explanation for the co-existence of IL-17A and IL-25 in patients with AR.

Our findings were further supported by the expression of chemokines (CCL20, IL-8, and eotaxin) in primary HNECs after treating with IL-17 cytokines and dsRNA. CCL20 and IL-8 are produced by dendritic cells and neutrophils, and are responsible for the enhancement of innate immunity (29, 30), while eotaxin is recognized as the chemokine of eosinophils and is responsible for allergic adaptive immunity (24). In this study, we found that IL-17A and IL-17F, as well as dsRNA, could significantly enhance CCL20 and IL-8 expression, while IL-25 was able to significantly increase eotaxin production in primary HNECs. These results have further confirmed the opposing roles of IL-17A and IL-25 in the allergic response. However, no significant suppression of IL-25-induced eotaxin production by IL-17A or IL-17F was observed, which might provide another explanation for the IL-25-mediated allergic response in patients with AR. Although dsRNA alone had no effect on eotaxin expression in HNECs, it was synergistic with IL-25, confirming the central role of IL-25 in the virus-epithelial cell-mediated IL-25/TSLP/eotaxin pathway that initiates the allergic cascade.

Taken together, our findings provided evidence of opposing regulation of TSLP by IL-17A and IL-25 in epithelial cells. The finding that IL-25 is dominant to IL-17A, with respect to TSLP regulation in AR, might represent a critical mechanism for understanding AR pathogenesis that could be applied to the design of effective strategies for future treatment.

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