

Thymic stromal lymphopoietin-induced human asthmatic airway epithelial cell proliferation through an IL-13-dependent pathway

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Background: Thymic stromal lymphopoietin (TSLP) plays a pivotal role in the initiation of allergic airway inflammation. This cytokine is produced by several cell types, including human epithelial cells.

Objective: We sought to determine the effect of TSLP on proliferation and repair of epithelial cells isolated from asthmatic patients and healthy subjects.

Methods: Expression of TSLP receptor (TSLPR) and its response to inhaled corticosteroids was evaluated on bronchial biopsy specimens of healthy control subjects and asthmatic patients by means of immunohistochemistry. TSLPR, TSLP, and IL-13 mRNA expression was determined by means of quantitative PCR, and protein expression was measured by means of ELISA and Western blotting in epithelial cells isolated from asthmatic subjects compared with those isolated from healthy control subjects. The effect of TSLP on cell proliferation and wound healing was performed.

Results: TSLPR is expressed by bronchial epithelial cells in bronchial biopsy specimens and in cultured cells, with no difference between asthmatic patients and healthy control subjects. Inhaled corticosteroids did not affect this expression. TSLP mRNA and protein levels were significantly higher in epithelial cells isolated from asthmatic patients compared with those from healthy control subjects. TSLP stimulated IL-13 production by bronchial epithelial cells. TSLP induced airway epithelial cell proliferation and enhanced epithelial injury repair. This effect was abrogated with IL-13 neutralization. **Conclusions:** Our data indicate that epithelial cells express TSLPR and that TSLP induces bronchial epithelial cell proliferation and increases injury repair through IL-13 production. This suggests that TSLP and IL-13 loops play a homeostatic role on epithelial cell proliferation and repair. (J Allergy Clin Immunol 2010;■■■:■■■-■■■.)

Key words: Asthma, cytokines, engineered bronchial tissue, epithelial cells

Allergic asthma is characterized by airway hyperresponsiveness and inflammation.^{1,2} This inflammation is the result of a complex immunologic cascade that leads to dysregulated production of T_H2 cytokines, such as IL-4, IL-5, and IL-13.^{3,4} Asthma is also characterized by structural changes in the bronchial mucosa leading to airway remodeling. Epithelial damage is one of the many pathophysiological features observed in asthma. It is believed that damage to the bronchial epithelium results in release of cytokines and growth factors that might affect epithelial cell function.

TSLP is produced by several cell types, including human epithelial cells and stromal and muscular cells.^{5,6} It was first identified as a growth factor for B cells in a thymic stromal cell line.⁷⁻⁹ This cytokine presents a structural and functional homology to IL-7.⁸ TSLP receptor (TSLPR) is a heterodimer consisting of a specific TSLPR and the IL-7 receptor α chain (IL-7R α).^{10,11} By interacting with the heterodimeric receptor IL-7R α /TSLPR, TSLP appears to initiate signal transduction through signal transducer and activator of transcription (STAT) 3 and STAT5 activation.¹⁰⁻¹⁴

Previous studies have demonstrated that TSLP plays a pivotal role in the pathology of allergic asthma and atopic dermatitis.⁵ Murine models of allergic asthma show higher TSLP expression in the lungs, whereas TSLPR-deficient mice are protected against the disease.¹⁵ Lung-specific overexpression of TSLP can induce hyperreactivity and T_H2-typed inflammation.¹⁶ In human subjects Ying et al¹⁷ have shown through *in situ* hybridization that TSLP is overexpressed in asthmatic bronchial biopsy specimens, and this correlates with T_H2-attracting chemokine levels. In normal human bronchial epithelial cells TSLP was upregulated by stimulation with Toll-like receptor 3 and T_H2 cytokines (IL-4 and IL-13), and this response was suppressed by glucocorticoid treatment.¹⁸ The regulation and function of epithelial TSLP expression has not been extensively studied. In this study we evaluated the expression of TSLPR on epithelial cells of asthmatic patients compared with those of healthy control subjects and studied the effect of TSLP on proliferation and repair of epithelial cells isolated from asthmatic patients and healthy subjects.

METHODS

Bronchial biopsy specimens

Bronchial biopsy specimens were obtained from "Banque de Tissus du Réseau en Santé Respiratoire du FRSQ, site Institut Universitaire de

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Abbreviations used

EGF: Epidermal growth factor
 EGFR: Epidermal growth factor receptor
 IL-7R α : IL-7 receptor α chain
 STAT: Signal transducer and activator of transcription
 TSLP: Thymic stromal lymphopoietin
 TSLPR: Thymic stromal lymphopoietin receptor

Cardiologie et de Pneumologie de Québec." Tissues from 10 asthmatic patients before and after 2 months of inhaled corticosteroid treatment and 10 healthy control subjects were used. Nonsmoking asthmatic patients had stable mild asthma, used only bronchodilators to treat their asthma symptoms, and were not exposed to sensitizing allergens; none of the study participants had an asthma exacerbation or respiratory tract infection in the preceding 4 weeks before entry into the study. No subjects had previously ever been treated with leukotriene modifiers or oral glucocorticosteroids in the previous 6 months or with nasal or dermal glucocorticosteroids within 30 days before their participation in the study. Bronchial biopsy specimens were taken at baseline and after treatment with 2 inhalations of 250 μ g of fluticasone dipropionate twice a day for 8 weeks. Subjects' characteristics are shown in Table I. All subjects provided written informed consent, and the study was approved by the Ethics Committee of Institut Universitaire de Cardiologie et de Pneumologie de Québec.

Immunohistochemistry

The alkaline phosphatase/antialkaline phosphatase procedure was used as previously described¹⁹ with a murine anti-human TSLPR (R&D system, Minneapolis, Minn). For negative control preparations, the primary antibody was replaced by Tris-buffered saline or the irrelevant isotype and species-matched primary antibody. The intensity of TSLPR staining was compared between slides processed simultaneously for immunostaining, as described previously.²⁰ Precautions were taken to ensure identical conditions of immunostaining for all slides that were to be compared in terms of time of antibody incubation, washing, developing, and counterstaining steps. The intensity of labeling was evaluated in a blind manner by 2 independent investigators and graded by using a 5-scale system (0, no signal; 1, weak; 2, moderate; 3, strong; 4, very strong; and 5 extremely strong), as previously described.²¹ A second evaluation several weeks later yielded identical values.

Epithelial cell culture

Epithelial cells were isolated from bronchial biopsy specimens of asthmatic patients (n = 6) and healthy control subjects (n = 7). Subjects were recruited from the Asthma Clinic at Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec, Canada). The ethics committee board approved the study, and all subjects provided written informed consent. The asthmatic patients were given diagnoses according to the American Thoracic Society criteria (methacholine PC₂₀, <4 mg/mL) and used only inhaled β_2 -agonists on demand.²² Their mean FEV₁ was 85% $\pm$ 3.1%, and their mean PC₂₀ was 4.0 $\pm$ 2.2 mg/mL. None used inhaled or systemic corticosteroids. All asthmatic patients were nonsmokers and atopic and had positive skin test results for house dust. Healthy control subjects (mean FEV₁ 98.2% $\pm$ 5.1% and mean PC₂₀ 99.4 $\pm$ 1.2 mg/mL) were nonatopic nonsmokers with no history of asthma or systemic diseases.

Epithelial cells were characterized by means of immunofluorescence and flow cytometry with anti-cytokeratin antibody from Calbiochem (San Diego, Calif), as previously described.^{23,24} Near-confluent epithelial cells from asthmatic patients and control subjects were detached and cultured in 6-well plates. Cells were cultured in 1% FBS-supplemented medium for 24 hours before experiments. Cells were then stimulated with IL-13 (10 ng/mL; eBioscience, San Diego, Calif), TNF (10 ng/mL; Peprotech, Rocky Hill, NJ), TSLP (1–50 ng/mL, R&D Systems), anti-IL-13 antibody (10 μ g/mL, R&D

Systems), and/or dexamethasone (10^{−7} mol/L; Sigma Chemicals, St Louis, Mo) from 2 to 24 hours and collected.

Real-time RT-PCR analysis

Total RNA and cDNA were prepared as previously described.²⁴ DNA was amplified by means of quantitative PCR, as previously described.²⁵ TSLPR was amplified by using the following primers: 5'-GAGTGGCAGTCCAAA CAGGAA-3' as sense and 5'-ACATCCTCCATAGCCTTACC-3' as antisense. IL-7R α was amplified by using the following primers: 5'-TGGACG CATGTGAATTTATC-3' as sense and 5'-CATTCATCCAGAAGCCTTT-3' as antisense. TSLP was amplified by using the following primers: 5'-TAT GAGTGGGACCAAAAAGTACCG-3' as sense and 5'-GGGATTGAAGGT TAGGCTCTGG-3' as antisense. IL-13 was amplified by using the following primers: 5'-ACCCAGAACCAGAAGGCTCCG-3' as sense and 5'-TCAGTT GAACCGTCCCTCGCG-3' as antisense. Data were acquired and analyzed with the computer software Opticon Monitor (version 2.02.24). Normalized expressions were calculated by using the calculated efficiency of each PCR reaction with a cDNA standard curve.

Immunofluorescence

Epithelial cells were grown to half confluence over 24 hours and fixed in 4% paraformaldehyde. Slides were blocked with rat serum and stained with a murine anti-human TSLPR (R&D Systems) or a murine anti-human IL-7R α (BD PharMingen, San Jose, Calif). Cell nuclei were stained with 1 μ g/mL Hoechst (Invitrogen, Carlsbad, Calif) for 10 minutes. Slides were mounted with the ProLong Antifade kit (Molecular probes, Eugene, Ore) to stabilize fluorescence. As a negative control, the irrelevant isotype antibody was used.

Western blots

Western blots were performed as described previously.²⁴ The following antibodies were used: murine anti-human TSLPR was from R&D Systems; murine anti-human IL-7R α , murine anti-human STAT3, and murine anti-human STAT5 were from BD PharMingen; rabbit anti-human phosphorylated STAT3 and rabbit anti-human phosphorylated STAT5 were from Cell Signaling (Danvers, Mass); and murine anti-human β -actin was from Sigma Chemicals.

ELISA

TSLP and IL-13 in culture supernatants were detected by means of ELISA (R&D Systems), according to the manufacturer's instructions. Calculated TSLP and IL-13 concentrations were normalized to cell numbers after stimulation. The minimum detectable levels of TSLP and IL-13 were 3.46 pg/mL and 32 pg/mL, respectively.

Cell proliferation assay

Cells were seeded at 3 $\times$ 10⁵ cells in 6-well plates, serum starved for 1 day, and treated with TSLP or IL-13 at 10 ng/mL with or without neutralizing anti-IL-13 antibodies. Isotype mouse IgG_k antibodies were used as negative controls (BD Biosciences, Mississauga, Ontario, Canada). All doses were previously determined with a dose-response study. After 4 days, cells were collected and counted. Cell counts were performed double-blind and performed twice by 2 independent individuals.

Monolayer wound repair assay

Epithelial cells were grown to confluence in 6-well plates and epidermal growth factor (EGF) starved. On confluence, wounds were made in the confluent monolayer of each well with a 200- μ L pipette tip. TSLP-treated cells, anti-IL-13-treated cells, or both were compared with nontreated cells 3, 6, or 12 hours after wounding. EGF (5 ng/mL) was used as a positive control. Several pictures at the same magnification were taken for each wound with a digital camera (Coolpix 950; Nikon, Tokyo, Japan). Wound distances were measured directly on the digital pictures in centimeters, and the percentage of

TABLE 1. Characteristics of healthy control subjects and asthmatic patients before and after corticosteroid treatment

	Healthy control subjects	Asthmatic patients	
		Before	After
Sex (M/F)	6/4	5/5	5/5
Age (y)	24.1 ± 4.30	24.7 ± 5.07	24.8 ± 5.15
FEV ₁ (L)	4.04 ± 0.70	3.67 ± 0.93	3.75 ± 0.82
FEV ₁ (%)	98.7 ± 9.05	95.8 ± 20.95	98 ± 16.50
PC ₂₀ (mg/mL)	144 ± 70.92	1.68 ± 1.84	3.83 ± 4.01

Values are presented as means ± SDs.

wound closure was calculated by comparing relative wound areas with wounds before stimulation.

Statistical analysis

All experiments in this study were performed at least 3 times. The data were analyzed with a crossed-nested design to compare groups and conditions. *A posteriori* multiple comparisons were performed with the Tukey-Kramer test. Mean values ± SEMs of quantitative variables were used as representative measures. All reported *P* values were 2-sided and were declared significant at .05 levels.

RESULTS

TSLPR expression in bronchial biopsy specimens

Immunohistochemical analysis of bronchial biopsy specimens showed that TSLPR is expressed in the epithelium and in bronchial mucosal layers of asthmatic patients and healthy control subjects (Fig 1, A-C). The score of immunostaining showed no significant difference between TSLPR (Fig 1, A-C) staining in asthmatic patients and healthy control subjects. Corticosteroid treatment of asthmatic patients showed no significant difference in immunostaining scores (Fig 1, A and B). Isotype and negative controls (Fig 1, D and E) showed no staining.

TSLPR is constitutively expressed in epithelial cells from asthmatic patients and cells from healthy control subjects

To assess whether epithelial cells express TSLPR, we determined the baseline expression of the specific TSLPR gene and the common IL-7R α gene with epithelial cells from asthmatic patients and control cells using real-time PCR. Both TSLPR and IL-7R α mRNA were expressed in epithelial cells from both control subjects and asthmatic patients (1.00 ± 0.33 vs 0.83 ± 0.22 TSLP fold induction [$P = .34$] and 1.03 ± 0.40 vs 1.65 ± 0.61 IL-7R α fold induction [$P = .21$], respectively; $n = 6$; Fig 2, A). We tested 2 cell lines (COS-7 and LnCAP) that express low levels of TSLPR. These cells served as negative controls. Moreover, we included monocytes as a positive control.

Immunofluorescence staining shows that bronchial epithelial cells express TSLPR protein at their surface (Fig 2, B, e). Fig 2, B, f, shows cytokeratin staining that indicates that all the cells are positive for this epithelial specific marker. Western blot analyses of epithelial cells from both asthmatic patients and control subjects showed that both IL-7R α and TSLPR proteins are expressed in these cells (Fig 2, C). After 10 minutes of TSLP treatment, both STAT3 and STAT5 were phosphorylated in epithelial cells of control subjects and asthmatic patients (Fig 2, D).

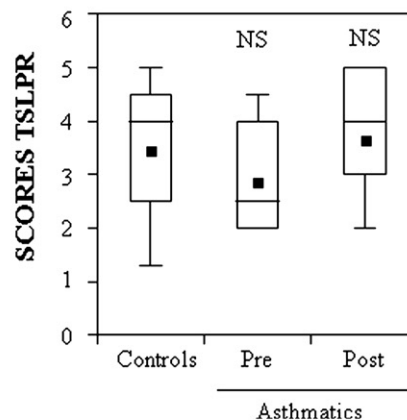
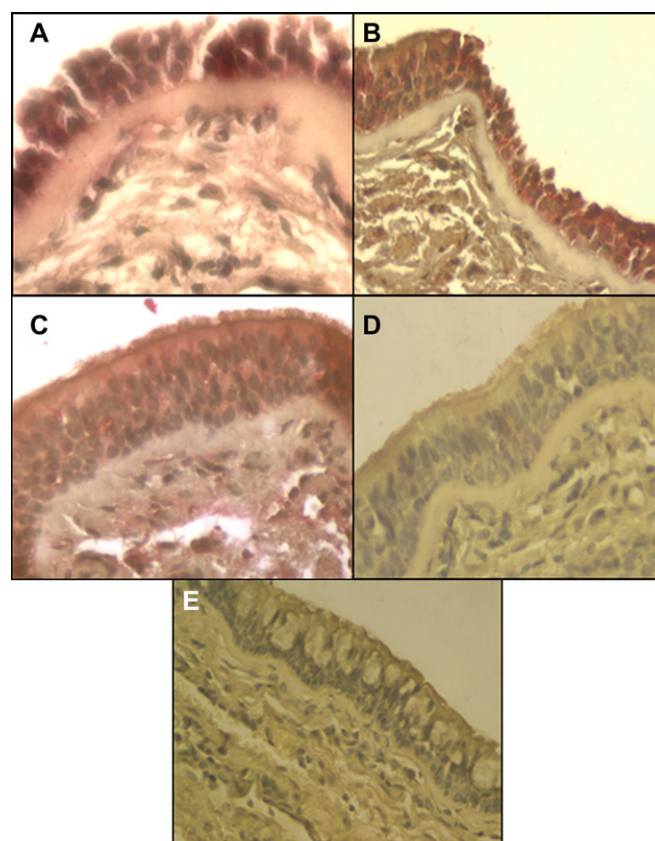


FIG 1. Immunohistochemistry staining of TSLPR. Representative staining of TSLPR in bronchial biopsy specimens of asthmatic patients before (A) and after 8 weeks of inhaled corticosteroid treatment (B), healthy control subjects (C), isotype controls (D), and negative controls (E). Individual scores of TSLPR immunoreactivity are represented in the lower panel ($n = 10$). NS, Not significant.

TSLP expression in bronchial epithelial cells from asthmatic patients

We compared the baseline expression of TSLP in epithelial cells isolated from control subjects and asthmatic patients. TSLP mRNA levels were higher in cells from asthmatic patients than in control cells (4.98 ± 0.87 vs 1.00 ± 0.64 fold induction of normal epithelial cell expression [$P = .006$], $n = 4$; Fig 3, A). TSLP protein expression was assessed in cell-culture supernatants. Concentrations of TSLP in cell-culture supernatants were

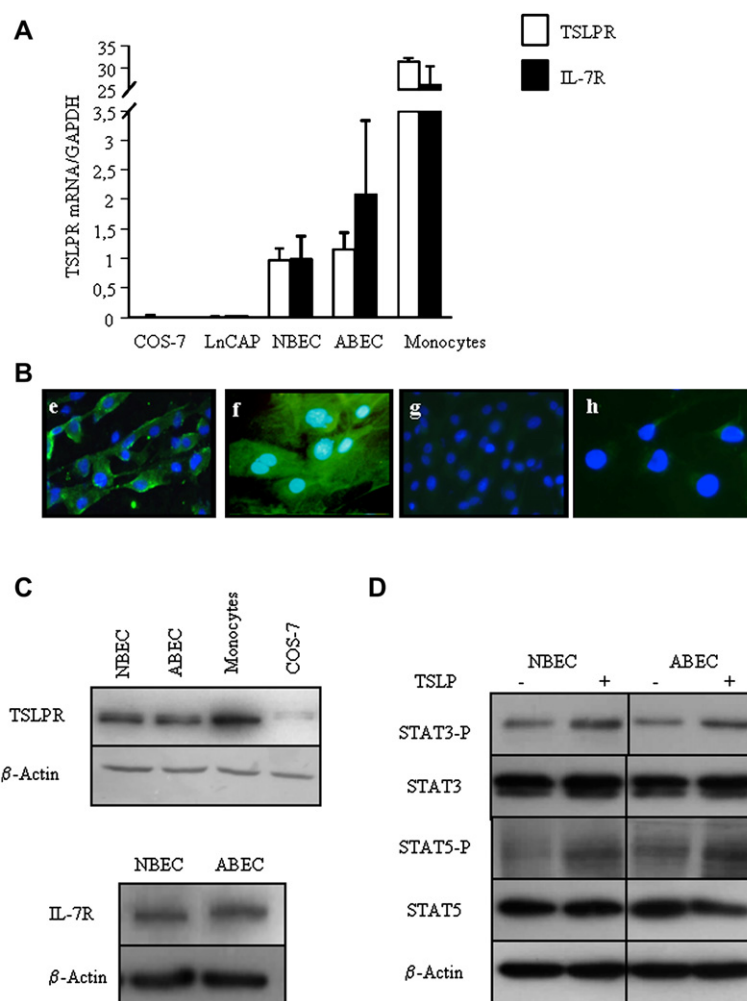


FIG 2. TSLPR and IL-7R α expression in bronchial epithelial cells. **A**, Real-time PCR analysis of TSLPR or IL-7R α expression on COS-7, LNCaP cell lines and on primary bronchial epithelial cells isolated from control subjects (NBEC) or asthmatic subjects (ABEC), and monocytes. **B**, Representative immunofluorescence staining of TSLPR (*e*) and cytokeratin in epithelial cells (*f*), negative isotype control (*g*), and TSLPR in COS-7 cells (*h*). Cell nuclei are stained in blue. **C**, Representative Western blots of TSLPR, IL-7R α , and β -actin. **D**, STAT3 and STAT5 phosphorylation after 10 minutes of stimulation with 10 ng/mL TSLP.

normalized to the number of cells in each well after stimulation. The baseline production of TSLP in epithelial cells from asthmatic patients was higher compared with that seen in cells from healthy control subjects (76.9 ± 3.4 vs 43.6 ± 2.9 pg/mL [$P = 10^{-4}$], $n = 5$; Fig 3, B).

Control epithelial cells and epithelial cells from asthmatic patients were stimulated with TNF or IL-13 for 6 hours, and TSLP mRNA was amplified by means of real-time PCR. Optimal conditions for IL-13 stimulation were previously validated with dose-response and time-course studies (data not shown). As shown in Fig 3, C, stimulation with TNF at different doses (from 1 to 100 ng/mL) and at different time points had no effect on TSLP mRNA expression in both types of epithelial cells. However, TSLP mRNA levels were significantly increased after IL-13 stimulation in both control cells (2.78 ± 0.34 vs 1.01 ± 0.01 fold induction [$P = 10^{-4}$], $n = 7$) and cells from asthmatic patients (12.72 ± 1.42 vs 4.35 ± 0.33 fold induction [$P = 10^{-4}$], $n = 5$). Treatment with dexamethasone significantly suppressed baseline expression of TSLP (0.41 ± 0.17 fold induction [$P = 10^{-5}$] in control epithelial cells and 0.56 ± 0.22 fold induction

[$P = .001$] in epithelial cells from asthmatic patients) and IL-13-induced TSLP expression in both control cells and cells from asthmatic patients (0.42 ± 0.18 fold induction [$P = 10^{-11}$] and 1.15 ± 0.41 fold induction [$P = .003$], respectively; Fig 3, C).

TSLP stimulated IL-13 production

We observed that epithelial cells from both control subjects and asthmatic patients increased their IL-13 production after TSLP stimulation in a dose-dependent manner (2.52 ± 0.52 fold induction [$P = .03$] and 1.87 ± 0.39 fold induction [$P = .02$] with nonstimulated cells, respectively; Fig 4, A). IL-13 was also detected in epithelial cell supernatants (Fig 4, B). Epithelial cells isolated from asthmatic patients produced more IL-13 than those from healthy control subjects at baseline (145.5 ± 6.1 and 92.7 ± 5.3 pg/mL, respectively; $P < .001$). TSLP stimulation significantly increased IL-13 protein secretion in both groups (241.1 ± 6.5 pg/mL [$P < .01$] in cells from healthy control subjects and 313.3 ± 12.6 pg/mL [$P < .01$] in cells from asthmatic

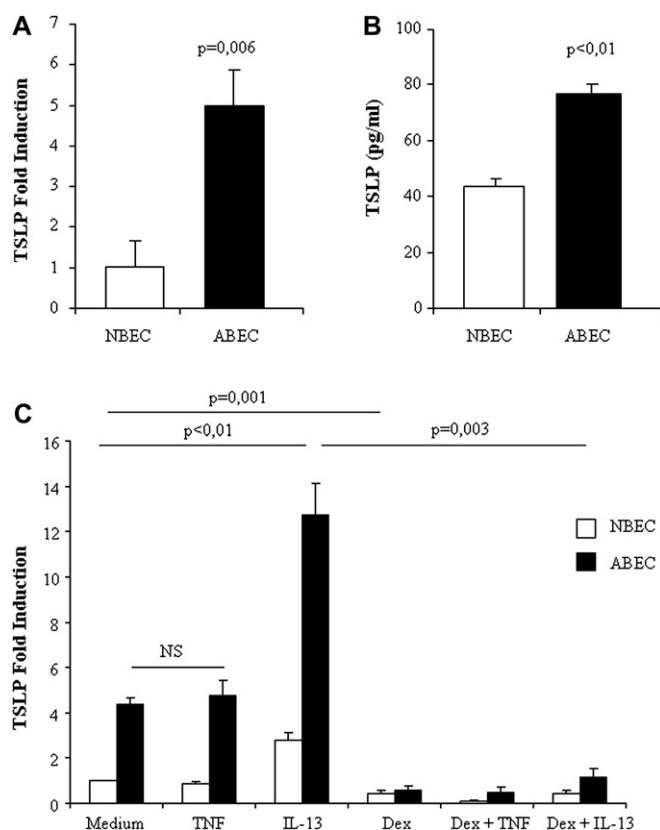


FIG 3. TSLP expression in bronchial epithelial cells from control subjects (NBEC) and asthmatic patients (ABEC). **A**, Real-time PCR analysis of TSLP expression. **B**, TSLP production by means of ELISA. **C**, Cells isolated from NBECs or ABECs were stimulated with IL-13 (10 ng/mL), TNF (10 ng/mL), and/or dexamethasone (Dex; 10^{-7} mol/L). Real-time PCR analysis of TSLP was performed. NS, Not significant.

patients). The effect of IL-13 production is more important in asthmatic patients than in healthy control subjects ($P = .001$).

Effect of TSLP on epithelial cell proliferation

Epithelial cells from asthmatic patients proliferated less than epithelial cells from healthy control subjects after 4 days of culture (3.08 ± 0.30 vs $1.51 \pm 0.01 \times 10^5$ cells, $P = .0002$). Fig 4, C, shows that TSLP induced significant epithelial cell proliferation in both control cells ($5.24 \pm 0.16 \times 10^5$ cells, $P < .01$) and cells from asthmatic patients ($2.41 \pm 0.39 \times 10^5$ cells, $P < .01$). TSLP-induced proliferation was comparable with IL-13 and EGF in both cell types. Addition of IL-13 neutralizing antibodies but not isotype control antibodies was able to reduce TSLP-induced epithelial cell proliferation to baseline ($3.18 \pm 0.41 \times 10^5$ cells [$P < .01$] in control cells and $1.52 \pm 0.06 \times 10^5$ cells [$P < .01$] in cells from asthmatic patients). IL-13 add-back experiments after neutralizing antibodies completely restored proliferation, suggesting that TSLP-induced proliferation is dependent of IL-13.

TSLP mediates airway epithelial layer wound closure

Epithelial cells from control subjects and asthmatic patients were stimulated with TSLP immediately after injury. Baseline wound closure was reduced in epithelial cells from asthmatic

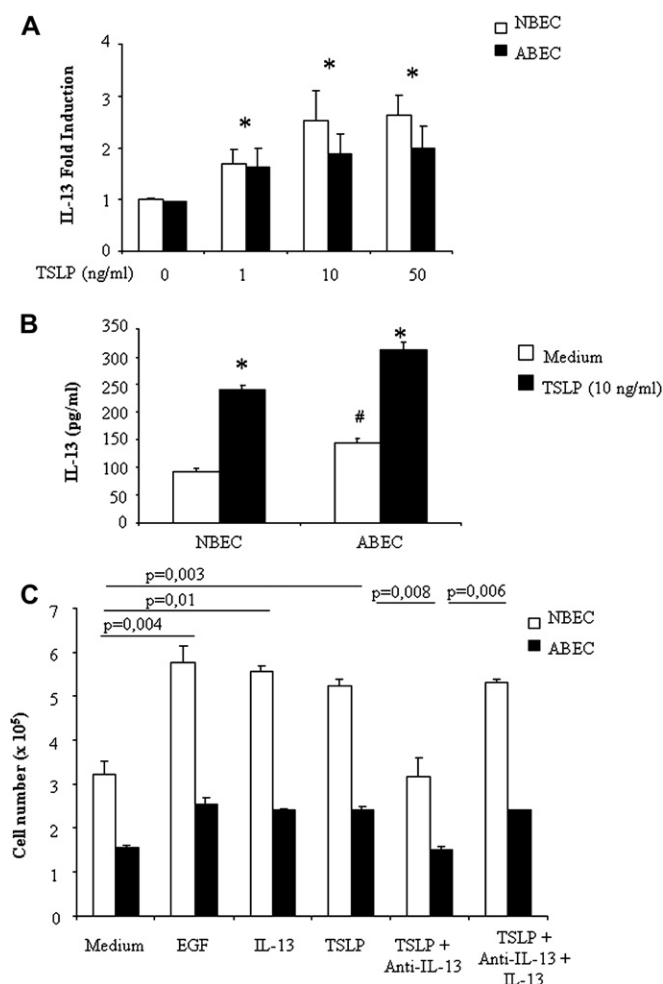


FIG 4. Effect of TSLP on IL-13 expression and cell proliferation. **A**, Cells isolated from control subjects (NBEC) and asthmatic patients (ABEC) were stimulated with different doses of TSLP. Real-time PCR analysis of IL-13 was performed. * $P < .05$ compared with respective unstimulated cells from control subjects and asthmatic patients. **B**, IL-13 ELISA after TSLP stimulation. * $P < .01$ versus unstimulated cells; # $P < .001$ between baseline NBECs and ABECs; ** $P = .001$ versus TSLP-stimulated NBECs. **C**, NBECs and ABECs were incubated with TSLP (10 ng/mL), IL-13 (10 ng/mL), and/or IL-13 neutralizing antibodies (10 μ g/mL) for 4 days, and proliferation was evaluated based on cell counts.

patients compared with that seen in cells from healthy control subjects ($30.3\% \pm 5.2\%$ and $61.5\% \pm 1.5\%$ wound closure at 6 hours, respectively; $P = .01$; Fig 5). At baseline, total closure of the wound was reached 12 hours after wounding in cells from healthy control subjects, whereas in asthmatic patients only 40% of the wound was closed. TSLP significantly induced wound closure of bronchial epithelium in healthy control subjects and asthmatic patients ($91.2\% \pm 4.4\%$ and $59.0\% \pm 2.6\%$ wound closure at 6 hours respectively; $P = .003$) with a comparable effect to EGF. However, the closure was not complete in cells from asthmatic patients compared with control cells. IL-13 neutralizing antibodies were able to completely inhibit TSLP-induced wound closure in both control cells and cells from asthmatic patients.

DISCUSSION

In this study we investigated the effect of TSLP on epithelial cell proliferation and wound healing. To our knowledge, this is the

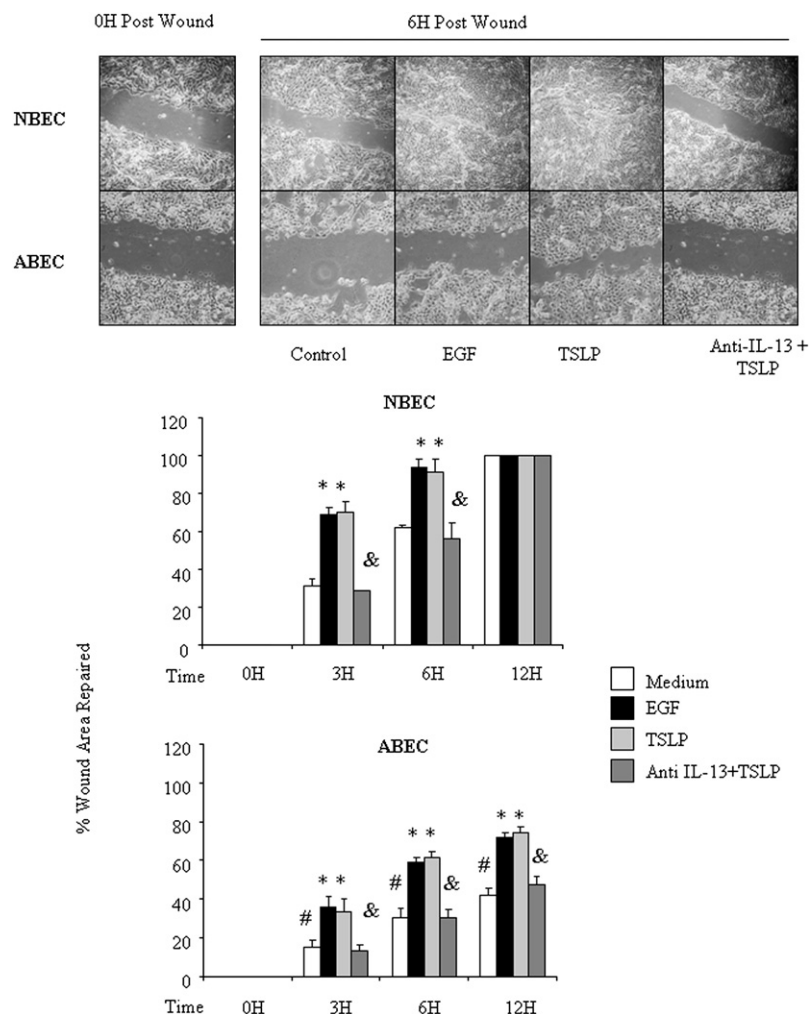


FIG 5. Effect of TSLP on epithelial cell repair. Confluent cells were stimulated with TSLP, EGF, and/or IL-13 neutralizing antibodies after mechanical injury. Examples of wound areas are shown in the upper panels, and calculated percentages of wound closures are shown in the bottom panel. * $P < .01$ versus unstimulated cells from control subjects; & $P < .05$ versus TSLP-stimulated cells; # $P < .05$ versus normal cell baselines.

first comparative study of the expression and function of TSLP and its receptor between bronchial epithelial cells isolated from bronchial biopsy specimens obtained from well-characterized asthmatic patients and healthy subjects. We found that epithelial cells from asthmatic patients overexpress TSLP. This concurs with the study by Ying et al,¹⁷ who found increased TSLP expression in asthmatic bronchial biopsy specimens. We also found that epithelial cells express TSLPR both in bronchial biopsy specimens and in cultured cells. This receptor activated STAT3 and STAT5 phosphorylation on stimulation with TSLP. We then examined the ability of T_H2 cytokines to regulate TSLP production in human airway epithelial cells. We found that IL-13 was capable of inducing TSLP production and that corticosteroids were able to reduce this production in both asthmatic and control cells. TSLP expression in control cells was also increased after exposure to IL-13. This suggests that the baseline increased TSLP expression observed in asthmatic cells might be due to the presence in their microenvironment of T_H2 cytokines.

Recently, it has been reported that epithelial cells can produce TSLP.¹⁸ This cytokine produced by epithelial cells and keratinocytes has been reported to modulate mature $CD4^+$ T-cell

proliferation and survival through its interaction with dendritic cells.¹⁵ TSLP stimulates T_H2 cytokine production, such as IL-13, by epithelial cells that can activate mast cells.²⁶ We showed that TSLP upregulates IL-13 expression on both control cells and cells from asthmatic patients. IL-13 is a T_H2 -like cytokine that has been considered a central mediator of airway remodeling in asthma.²⁷ Epithelial cells have been shown to produce IL-13 that in turn induced their proliferation.^{28,29} In the present study we showed that TSLP stimulated IL-13 production by epithelial cells. This induction is more important in cells from asthmatic patients than control cells. IL-13 in turn induced proliferation and increases wound closure. In cells from asthmatic patients, the effect of TSLP on proliferation and wound healing is still limited compared with that seen in cells from healthy control subjects. This might be explained by the fact that other cytokines and growth factors present in the epithelial cell environment might also affect these epithelial cell functions. Bronchial epithelial cells can produce an array of proinflammatory mediators, growth factors, and cytokines in response to environmental challenges.³⁰⁻³³ All these factors can affect their proliferation and repair.^{34,35} Stimulation with TSLP induced bronchial epithelial cell proliferation and

increased injury repair through IL-13 production. We showed that this effect is comparable with the effect of EGF, a well-known growth factor for epithelial cells. Several studies showed a relationship between IL-13 and the EGF receptor (EGFR) pathway in which EGFR activation is necessary for epithelial repair.^{29,36} We have previously shown that in epithelial cells from asthmatic patients, EGFR pathways are disturbed mainly because of the presence of a more active form of TGF- β in cells from asthmatic patients than cells obtained from healthy subjects.²⁴ This might explain that despite the higher production of TSLP and IL-13 by epithelial cells from asthmatic patients, they were not able to proliferate and repair in the same rate of control cells.

In conclusion, this study suggests that TSLP, in addition to its function on inflammatory cells, can be considered a homeostatic cytokine that regulates proliferation and injury repair in airway epithelium through an IL-13 retro-control loop.

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Key message

- TSLP induces bronchial epithelial cell proliferation and repair through IL-13 production, which suggests a new homeostatic role for this cytokine.

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