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Expression and Cellular Provenance of Thymic Stromal Lymphopoietin and Chemokines in Patients with Severe Asthma and Chronic Obstructive Pulmonary Disease¹

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Asthma and chronic obstructive pulmonary disease (COPD) are associated with Th2 and Th1 differentiated T cells. The cytokine thymic stromal lymphopoietin (TSLP) promotes differentiation of Th2 T cells and secretion of chemokines which preferentially attract them. We hypothesized that there is distinct airways expression of TSLP and chemokines which preferentially attract Th1- and Th2-type T cells, and influx of T cells bearing their receptors in asthma and COPD. In situ hybridization, immunohistochemistry, and ELISA were used to examine the expression and cellular provenance of TSLP, Th2-attracting (TARC/CCL17, MDC/CCL22, I-309/CCL1), and Th1-attracting (IP-10/CXCL10, I-TAC/CXCL11) chemokines in the bronchial mucosa and bronchoalveolar lavage fluid of subjects with moderate/severe asthma, COPD, and controls. Cells expressing mRNA encoding TSLP, TARC/CCL17, MDC/CCL22, and IP-10/CXCL10, but not I-TAC/CXCL11 and I-309/CCL1, were significantly increased in severe asthma and COPD as compared with non-smoker controls ($p < 0.02$). This pattern was reflected in bronchoalveolar lavage fluid protein concentrations. Expression of the same chemokines was also increased in ex- and current smokers. The cellular sources of TSLP and chemokines were strikingly similar in severe asthma and COPD. The numbers of total bronchial mucosal T cells expressing the chemokine receptors CCR4, CCR8, and CXCR3 did not significantly differ in asthma, COPD, and controls. Both asthma and COPD are associated with elevated bronchial mucosal expression of TSLP and the same Th1- and Th2-attracting chemokines. Increased expression of these chemokines is not, however, associated with selective accumulation of T cells bearing their receptors. *The Journal of Immunology*, 2008, 181: 2790–2798.

Severe asthma and chronic obstructive pulmonary disease (COPD)³ are characterized clinically by similar symptoms, episodes of exacerbation, and variable airways obstruction. These phenomena are thought to reflect airways inflammation. COPD and asthma are conventionally described as “Th1/macrophage/neutrophil” and “Th2/eosinophil” mediated, respectively (1, 2). In reality, however, there is a considerable degree of overlap (3–8).

Cellular infiltration in inflammation is postulated to be regulated by chemokines, which attract specific subsets of leukocytes according to their expression of chemokine receptors (9). In the case of T cells, the chemokines TARC/CCL17 and MDC/CCL22 are ligands for the chemokine receptor CCR4, expressed on Th2 cells, whereas I-309/CCL1 is a ligand for CCR8 expressed on a subset of these cells (9, 10). IP-10/CXCL10 and I-TAC/CXCL11 are ligands for CXCR3, expressed on Th1 cells (11). Although some studies have suggested accumulation of CCR4 expressing T cells in allergic inflammation (12, 13), in general T cell chemokine receptor expression seems to be associated more clearly with tissue distribution than with function (14).

Little is known about what regulates expression of chemokines at mucosal surfaces in vivo, but attention has recently been drawn to the possible role of the IL-7-like cytokine thymic stromal lymphopoietin (TSLP), which induces the production of Th2-attracting chemokines such as TARC/CCL17 and MDC/CCL22 by dendritic cells, which then also prime development of T cells producing Th2 cytokines (15). We recently reported increased expression of TSLP and TARC/CCL17 in asthmatic airways as compared with those of normal controls (16).

We have now investigated the specificity of these findings by comparing the expression of TSLP, the Th2-attracting chemokines TARC/CCL17, MDC/CCL22, and I-309/CCL1, and the Th1-attracting chemokines IP-10/CXCL10 and I-TAC/CXCL11 in the bronchial mucosa and bronchoalveolar lavage (BAL) fluid of subjects with moderate/severe asthma and COPD and control subjects with normal lung function with and without a current or prior smoking history. We reasoned that, if the bronchial mucosa in

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³ Abbreviations used in this paper: COPD, chronic obstructive pulmonary disease; TSLP, thymic stromal lymphopoietin; BAL, bronchoalveolar lavage; FEV₁, forced expiratory volume in the first second; ISH, in situ hybridization; IHC, immunohistochemistry.

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Table I. Clinical data on asthmatics, COPD, subjects, and controls^a

	Gender (F:M)	Age	FEV ₁ (% predicted)	Therapy ^b
Asthma (n = 13)	3:10	55 (31–73)	50.0 (31.5–79.9) ^c	LABA (n = 10/13) ICS (n = 13/13)
COPD	4:11			
Smoker (n = 7)		53 (45–72)	54.8 (32.3–72.2) ^c	LABA (n = 5/7) ICS (n = 3/7)
Ex-smoker (n = 8)		62 (51–75)	54.8 (39.4–71.4) ^c	LABA (n = 4/8) ICS (n = 5/8)
Control	13:17			
Never smoker (n = 10)		53 (41–68)	104.4 (91.2–131.0)	
Smoker (n = 10)		54 (32–71)	96.8 (86.8–127.5)	
Ex-smoker (n = 10)		61 (55–67)	100.0 (86.2–123.7)	

^a Data are expressed as the median (range).^b LABA, long-acting β_2 -agonist; ICS, inhaled corticosteroid.^c Pre-bronchodilator measurements, Kruskal Wallis test $p < 0.05$ between groups, Mann-Whitney U test $p = 0.0001$ vs controls.

asthma and COPD is populated by functionally distinct T cells, and that if chemoattraction by chemokines plays a significant role in this process, then we might be able to detect differential expression of TSLP, chemokines, and T cells expressing their receptors in each disease. We also examined the cellular provenance of some of these molecules in the bronchial mucosa. We hypothesized that differential airways expression of TSLP is associated with different patterns of chemokine expression in severe asthma and COPD, and that this in turn is reflected in differential infiltration of inflammatory cells, particularly functional subsets of T cells expressing ligands for these chemokines.

Materials and Methods

Subjects and fiberoptic bronchoscopy

Subjects were recruited in the Department of Asthma, Allergy and Respiratory Science, King's College London School of Medicine, U.K. The study was approved by the Ethics Committee of King's College Hospital, and each participant provided written, informed consent. Endobronchial biopsy specimens and BAL fluid were obtained at fiberoptic bronchoscopy from 13 patients with moderate/severe asthma (three of the patients were included in our previously published study) (16), 15 patients with COPD (seven smokers and eight ex-smokers), and 30 healthy controls (10 non-smokers, ex-smokers, and current smokers) (Table I). Asthmatic subjects had a history of typical symptoms, an obstructive ventilatory defect (forced expiratory volume in the first second (FEV₁) < 80% of the predicted value, with FEV₁/forced vital capacity ratio < 0.7), a median 21% (range 16–36%) improvement in FEV₁ following inhaled β_2 -agonist (nebulized salbutamol 2.5 mg), and

histamine PC₂₀ < 6 mg/ml measured in the 2 wk before the biopsy. None had ever smoked and there was no history of other respiratory disease. Atopy was defined as the presence of one or more positive skin prick tests to a range of common aeroallergens. Of the asthmatics, 11:13 were atopic. COPD was defined according to the Global initiative for chronic obstructive lung disease criteria as postbronchodilator FEV₁/forced vital capacity ratio < 0.7 (17). Subjects with COPD had a smoking pack-year history ≥ 15 , and showed a median 8% (range 1–13%) improvement in FEV₁ following inhaled bronchodilator. They had moderate or severe disease according to the Global initiative for chronic obstructive lung disease criteria. None had a prior history of other respiratory diseases, including asthma; all were non-atopic subjects and were clinically free of exacerbation and systemic glucocorticoid therapy for at least 1 mo before the study. Ex-smokers had abstained from smoking for > 1 yr. Age matched control subjects had no history of lung disease (Table I). Of the controls, 7:30 were atopic. Fiberoptic bronchoscopy was performed, and BAL fluid and bronchial biopsies were obtained and processed as previously described (16, 18).

In situ hybridization (ISH) and immunohistochemistry (IHC)

ISH was performed to identify mRNA encoding human TARC/CCL17, MDC/CCL22, TSLP, IP-10/CXCL10, I-TAC/CXCL11, and I-309/CCL1 using digoxigenin-labeled riboprobes as previously described (16, 18). Single IHC was used using monoclonal and polyclonal Abs to identify structural cells and specific inflammatory leukocytes within bronchial mucosal sections. The techniques and most of the reagents have been described in our previous studies (16, 18–20). To identify cells expressing CCR4, CCR8, and CXCR3, we used Abs purchased from Santa Cruz Biotechnology, Alexis Biochemicals, and R&D Systems, respectively. CCR4⁺ cells coexpressing CD4 and CD8 were identified using double IHC using protocols previously described (19). To identify the cellular sources of TSLP,

Table II. The numbers of inflammatory cells in the epithelium (per mm length of basement membrane) and submucosa (per mm² of submucosa) of bronchial biopsies from moderate/severe asthma, COPD, and controls^a

	CD4	CD8	CD68	Tryptase	MBP	Elastase
Epithelium						
Asthma (n = 13)	1.3 (0.0–6.0)	2.0 (0.0–8.0)	5.9 (0.0–9.2)	2.1 (0.0–7.8)	0.2 (0.0–2.9)	4.2 (0.0–13.3)
COPD						
Smoker (n = 7)	0.6 (0.0–4.6)	1.3 (0.0–4.7)	2.3 (0.0–6.5)	0.5 (0.0–2.6)	0.0 (0.0–2.0)	6.7 (0.0–21.2)
Ex-smoker (n = 8)	2.3 (0.0–24.0)	1.7 (0.0–11.3)	3.5 (0.0–8.4)	1.0 (0.0–4.1)	0.0 (0.0–0.5)	6.1 (2.3–21.2)
Control						
Never smoker (n = 10)	1.5 (0.0–5.4)	0.6 (0.0–5.0)	4.2 (2.1–7.9)	4.3 (1.1–7.7)	0.0 (0.0–1.2)	2.1 (0.0–7.8)
Smoker (n = 10)	2.0 (0.0–7.0)	1.8 (0.0–13.0)	3.3 (0.0–15.0)	2.4 (0.6–5.9)	0.0 (0.0–1.5)	3.0 (0.0–9.4)
Ex-smoker (n = 10)	1.0 (0.0–4.0)	0.5 (0.0–8.0)	1.8 (0.0–7.0)	3.5 (1.2–5.5)	0.0 (0.0–1.2)	3.1 (0.0–9.6)
Submucosa						
Asthma (n = 13)	18.7 (4.6–275.0)	20.3 (4.9–150.0)	40.5 (17.8–355.0)	22.7 (4.9–41.2)	7.0* (1.1–17.1)	20.3** (13.9–482.5)
COPD						
Smoker (n = 7)	18.6 (5.0–39.4)	20.1 (10.1–40.3)	36.3 (9.0–60.3)	10.2 (3.7–31.7)	1.1 (0.0–2.9)	34.9** (14.3–77.8)
Ex-smoker (n = 8)	12.7 (5.4–116.9)	21.1 (11.6–58.3)	34.3 (9.0–60.3)	10.9 (4.0–64.2)	1.8 (0.0–6.3)	21.8 (4.6–36.4)
Control						
Never smoker (n = 10)	10.5 (4.3–141.0)	8.5 (4.4–51.3)	18.7 (7.1–44.0)	17.9 (7.1–31.3)	0.6 (0.0–4.7)	6.1 (1.7–11.9)
Smoker (n = 10)	29.0 (1.6–104.5)	28.5 (1.0–67.5)	63.7 (17.0–92.5)	54.7 (15.3–142.8)	0.8 (0.0–5.6)	33.7** (7.3–233.3)
Ex-smoker (n = 10)	36.7 (2.2–58.1)	19.5 (2.4–43.2)	49.7 (7.9–82.3)	29.4 (6.6–184.6)	0.7 (0.0–6.7)	28.6** (6.6–103.5)

^a The data are expressed as the median (range).* $p < 0.01$ (vs COPD and all controls); ** $p < 0.05$ (vs never-smoker controls) (Mann-Whitney U test).

Table III. The numbers of CCR4, CCR8, and CXCR3 immunoreactive cells in the submucosa (per mm² of submucosa) of bronchial biopsies from moderate/severe asthmatics, COPD patients, and normal controls^a

	CCR4	CCR8	CXCR3
Asthma (n = 13)	19.5 (2.7–31.0)	4.1 (0.6–12.1)	16.9 (2.1–43.3)
COPD			
Smoker (n = 7)	15.2 (10.0–20.5)	4.4 (2.5–12.1)	21.0 (9.1–38.0)
Ex-smoker (n = 8)	16.5 (12.0–27.3)	7.4 (3.8–17.9)	18.8 (5.1–28.3)
Control			
Never smoker (n = 10)	17.5 (2.0–58.3)	4.3 (0.9–29.1)	22.3 (3.3–48.8)
Smoker (n = 10)	17.1 (6.3–22.8)	6.3 (1.1–17.5)	22.7 (2.0–28.3)
Ex-smoker (n = 10)	21.3 (3.3–33.6)	10.0 (0.0–15.0)	20.6 (2.0–33.7)

^a The data are expressed as the median (range).

TARC/CCL17, and IP-10/CXCL10 mRNA, sequential IHC/ISH was used as previously described (16). Slides were counted by two independent observers blind to the patients' clinical status, using an eyepiece graticule as previously described (16, 18). The mean \pm SD entire cross-sectional areas of the biopsy sections examined in each of the three groups (asthma, COPD, control) were 3.2 ± 0.3 , 3.6 ± 0.4 , and 3.7 ± 0.4 mm², respectively, with within-group coefficients of variation from 10.4 to 12.1%. The mean \pm SD basement membrane lengths counted were 4.3 ± 0.6 , 4.1 ± 0.4 , and 4.7 ± 0.6 mm, respectively, with within-group coefficients of variation from 17.6 to 19.1%. The between observer coefficients of variation for duplicate counts for all markers tested for all three groups varied from 2.2 to 3.9%.

ELISA for TSLP and chemokines

BAL fluid samples were concentrated 20 times using Amicon Ultra-15 filters (Millipore). Concentrations of human TARC/CCL17, MDC/CCL22, IP-10/CXCL10, I-TAC/CXCL11, and I-309/CCL1 in concentrated BAL fluid were measured using commercial ELISA kits according to the manufacturer's instructions (R&D Systems). Limits of detection were 7.8, 7.8, 31.25, 7.8, and 7.8 pg/ml, respectively. TSLP concentrations in BAL fluid were determined using an in-house ELISA developed by Novartis, with sensitivity 1.0 pg/ml. Samples were adjusted where necessary so that analyte concentrations fell within the linear range of the standard curves. The data were normalized to the total protein content of the fluid as determined by the bicinchoninic acid protein assay (Pierce) according to the manufacturer's instructions. To do this, all analyte concentrations were divided by the fold increase in total protein concentration in each BAL sample as compared with the sample with the lowest total protein concentration.

Statistical analysis

Data were analyzed with the aid of a commercially available statistical package (Minitab for Windows Release 9.2; Minitab Inc.). Significant variation in the data within groups was investigated using Kruskal Wallis ANOVA. The Mann-Whitney *U* test (with Bonferroni's correction) was used to compare variance between groups. Correlation coefficients were obtained by Spearman's rank-order method with correction for tied values. For all tests, $p < 0.05$ was considered significant.

Results

Clinical data

These are summarized in Table I. The median FEV₁ (% predicted) measurements in asthmatics and COPD patients were not significantly different, but both were significantly lower than those of the controls.

Inflammatory cellular infiltration and chemokine receptor expression

Single IHC showed that the numbers of CD4⁺ and CD8⁺ T cells, tryptase⁺ mast cells, MBP⁺ eosinophils, CD68⁺ macrophages, and elastase⁺ neutrophils were statistically similar in the bronchial epithelium in asthma, COPD, and controls (Table II). There were no differences in cell counts in the asthmatics and controls according to atopic status (data not shown). Compared with COPD patients and all of the control groups, the median number of MBP⁺

eosinophils was significantly increased in the submucosa of asthmatics ($p < 0.01$). The median number of elastase⁺ neutrophils was significantly elevated in the submucosa of subjects with asthma, COPD, and non-COPD smokers and ex-smoker controls as compared with never smoking controls ($p < 0.05$) (Table II). There were no significant differences in the numbers of cells expressing immunoreactivity for CCR4, CCR8, or CXCR3, either in the epithelium or in the submucosa, in the asthmatics, COPD patients, and the control groups (Table III). Double IHC performed on biopsies of a subset of subjects showed that there were no significant differences in the median percentages of CCR4⁺ cells coexpressing the T lymphocyte markers CD4 or CD8 in the submucosa of the biopsies from asthmatics and COPD smokers and ex-smokers (Table IV).

TSLP mRNA expression

Typical examples of single ISH and sequential IHC/ISH and double IHC are shown in Fig. 1. Control experiments using sense probes produced uniformly negative staining. Fig. 2 shows analysis of TSLP mRNA expression by single ISH. In the epithelium, the median numbers of TSLP mRNA⁺ cells were not significantly different in asthma and COPD (smokers and ex-smokers), but both were significantly elevated as compared with the non-smoking controls (Fig. 2, *top*). TSLP expression was also significantly increased in non-COPD smokers and ex-smokers as compared with non-smoker controls (Fig. 2, *top*) ($p = 0.0028$). A similar pattern was observed in the submucosa (Fig. 2, *bottom*) ($p = 0.0114$).

Th₂-attracting chemokine (TARC/CCL17, MDC/CCL22, and I-309/CCL1) mRNA expression

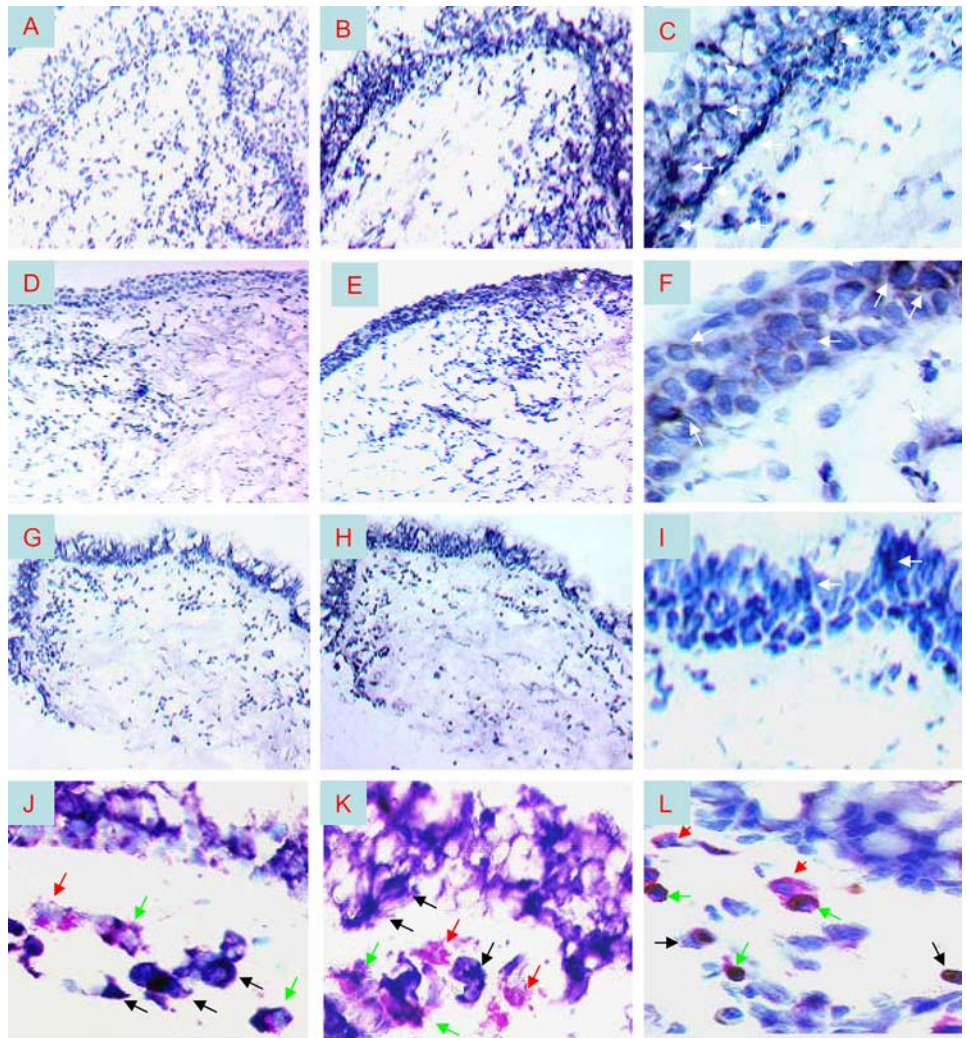
In the epithelium, median numbers of TARC/CCL17 and MDC/CCL22 mRNA⁺ cells were not significantly different in asthma and COPD but were significantly elevated as compared with the non-smoking controls (Fig. 3, *left, top, and middle*) (TARC/CCL17: $p = 0.0016$; MDC/CCL22: $p = 0.0009$ asthma vs non-smoker controls; $p = 0.0013$ and $p = 0.0012$ COPD vs non-smoker controls). A similar situation pertained in the

Table IV. The percentages of CCR4⁺ cells co-expressing CD4 or CD8 in the submucosa of bronchial biopsies from severe asthmatics and COPD patients (smoker and ex-smoker)^a

	CCR4 ⁺ /CD4 ⁺	CCR4 ⁺ /CD8 ⁺
Asthma (n = 6)	59.1 (55.6–67.8)	12.8 (3.7–20.0)
COPD		
Smoker (n = 6)	42.9 (20.0–64.1)	4.6 (0.0–14.2)
Ex-smoker (n = 6)	40.0 (14.3–75.0)	14.2 (0.0–30.1)

^a The data are expressed as the median (range).

FIGURE 1. Typical examples of single ISH, sequential IHC/ISH, and double IHC in bronchial biopsies. Single ISH with digoxigenin-labeled TSLP riboprobes in sections of bronchial biopsies from a patient with COPD (*top*, A–C), an asthmatic (*middle*, D–F), and a control subject (*bottom*, G–I). *Left panels* (A, D, G) show hybridization with a sense (control) riboprobe, *middle panels* (B, E, H) show hybridization with anti-sense riboprobe at low power (original magnification $\times 100$), and *right panels* (C, F, I) show the same sections at higher power (original magnification $\times 1000$). Positive cells stained blue black (highlighted by white arrows). Sequential IHC/ISH showing a single stained CD31⁺ endothelial cell (J) or neutrophils (K) (red, red arrows), single TSLP mRNA⁺ cells (blue/black, black arrows), and double stained cells (green arrows). L, Sequential double IHC showing single CD4⁺ cells (red, red arrows), single CCR4⁺ cells (brown, black arrows), and double stained cells (green arrows). The original magnifications are $\times 20$ for A, B, D, E, G, and H, whereas C, F, and I are at high power magnification ($\times 100$).



submucosa (Fig. 3, *right, top*, and *middle*). Increased expression of mRNA encoding TARC/CCL17 and MDC/CCL22 was observed in smokers and ex-smokers without COPD as compared with the non-smoking controls (TARC/CCL17: $p = 0.0018$, MDC/CCL22: $p = 0.0122$) (Fig. 3). This pattern of expression reflected that TSLP; indeed, the numbers of cells expressing TSLP mRNA correlated positively with the numbers of cells expressing TARC/CCL17 mRNA (epithelium: $r = 0.485$, $p = 0.009$; submucosa: $r = 0.442$, $p = 0.019$) and MDC/CCL22 mRNA (submucosa: $r = 0.42$, $p = 0.026$) although the total numbers of cells expressing TSLP mRNA were considerably lower than those expressing TARC/CCL17 and MDC/CCL22 mRNA. No significant differences in the numbers of I-309/CCL1 mRNA⁺ cells, either in the epithelium or the submucosa, were observed between the subjects with asthma and COPD and the controls regardless of smoking status (Fig. 3, *bottom*).

Th₁-attracting chemokine (IP-10/CXCL10 and I-TAC/CXCL11) mRNA expression

The median numbers of IP-10/CXCL10 mRNA⁺ cells were not significantly different in the patients with asthma and COPD, but both were significantly elevated, both in the epithelium and the submucosa, as compared with the non-smoking controls (Fig. 4, *top*). In addition, the median numbers of mRNA⁺ cells in smokers and ex-smokers were significantly increased as compared with non-smokers (Fig. 4, *top*). In contrast, there were no

significant differences in the median numbers of I-TAC/CXCL11 mRNA⁺ cells, either in the epithelium or in the submucosa, between the four subject groups (Fig. 4, *bottom*).

Cellular sources of TSLP, TARC/CCL17, and IP-10/CXCL10

Sequential IHC/ISH showed that in the epithelium of patients with asthma and COPD, a mean of more than 75% of the cells expressing mRNA encoding TSLP, TARC/CCL17, and IP-10/CXCL10 were cytokeratin⁺ epithelial cells. The percentages of TSLP and IP-10/CXCL10 mRNA⁺ cells accounted for by epithelial cells were slightly but significantly greater in COPD than in asthma ($p = 0.032$, 0.012 , respectively) (Fig. 5, *top*). Epithelial tryptase⁺ mast cells comprised slightly but significantly more of the TSLP and TARC/CCL17 mRNA⁺ cells in asthma as compared with COPD ($p = 0.04$, 0.016 , respectively). The remainder of the mRNA⁺ cells comprised of elastase⁺ neutrophils and CD68⁺ macrophages with very few CD3⁺ T cells and MBP⁺ eosinophils. In the submucosa, CD31⁺ endothelial cells, elastase⁺ neutrophils, tryptase⁺ mast cells, and CD68⁺ macrophages were the principal cellular sources of TSLP, TARC/CCL17, and IP-10/CXCL10 (Fig. 5, *bottom*). Submucosal tryptase⁺ mast cells constituted slightly but significantly higher percentages of the cells expressing TSLP and TARC/CCL17 mRNA ($p = 0.036$ in each case), and CD68⁺ macrophages contributed slightly but significantly higher percentages of the cells expressing TSLP and IP-10/

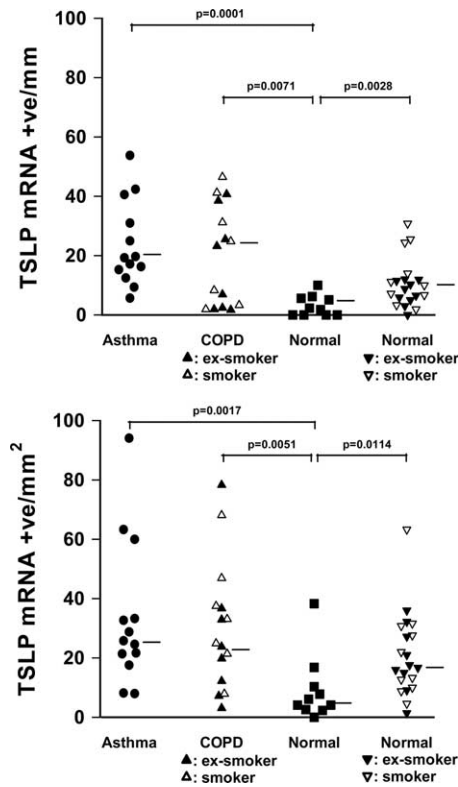


FIGURE 2. TSLP mRNA⁺ cells in bronchial biopsies. Numbers of cells expressing TSLP mRNA detected by ISH in the epithelium (per mm length of basement membrane) (*top*) and submucosa (per mm² of submucosa) (*bottom*) of bronchial biopsies from moderate/severe asthma ($n = 13$), COPD (smoker/ex-smoker = 7:8), and normal controls (never smoker/smoker/ex-smoker = 10:10:10). Kruskal-Wallis ANOVA (within groups) and Mann-Whitney U test (between groups).

CXCL10 ($p = 0.021, 0.012$, respectively) in asthma as compared with COPD.

Concentrations of TSLP and chemokines in BAL

Consistent with the pattern of mRNA expression in the bronchial mucosa, the median concentrations of TSLP were significantly elevated in BAL fluid from subjects with asthma and COPD compared with controls (Fig. 6, *left, top*; $p \leq 0.02$). Concentrations of TARC/CCL17 and MDC/CCL22 were also significantly higher in BAL fluid from subjects with asthma (TARC: $p = 0.0207$, MDC: $p = 0.027$) and COPD (TARC: $p = 0.048$, MDC: $p = 0.013$) compared with non-smoking controls (Fig. 6, *top, right, and middle left*). IP-10/CXCL10 was also elevated in asthmatics compared with non-smoking controls ($p = 0.027$) (Fig. 6, *middle right*). The median concentrations of TARC/CCL17, MDC/CCL22, and IP-10/CXCL10 were also increased in BAL fluid in control subjects with a smoking history as compared with never smokers, although in contrast with their mRNA expression these differences were not statistically significant. The concentrations of I-TAC/CXCL11 and I-309/CCL1 were undetectable or low in most of the BAL samples tested and not significantly different between the groups (Fig. 6, *bottom*).

Discussion

We present a study of the expression of TSLP and key proinflammatory chemokines in the airways of patients with well-defined asthma and COPD showing a similar degree of airways obstruction. As measured by both mRNA⁺ cells in the epithelium and submucosa of bronchial biopsies and protein concentrations in BAL fluid, expression of TSLP and the CCR4 ligands TARC/CCL17 and MDC/CCL22 was significantly increased in the airways of both groups of patients as compared with never-smoking controls. Bronchial epithelial and mucosal expression of mRNA encoding IP-10/CXCL10 was also significantly increased in both asthma and COPD, although this was reflected in significantly elevated BAL concentrations of the corresponding protein only in the asthmatics. Expression of I-TAC/CXCL11 and I-309/CCL1 did not differ in patients with asthma and COPD and controls however measured. One would not expect a tight correlation between mRNA expression as assessed by ISH and protein expression in BAL fluid because (i) ISH is semiquantitative and mRNA expression may not equate with secretion of the corresponding protein; (ii) there may be other sources of TSLP and chemokine synthesis, for example cells within the bronchial lumen and airways smooth muscle cells, recently reported as a potential source of TSLP in COPD patients (21); (iii) it has been reported (22) that TSLP mRNA expression in epithelial cells may or may not be associated with protein expression depending on the nature of the provoking stimulus. The mRNA and protein data are, however, mutually corroborative.

The data corroborate our previous study of TSLP expression in another group of asthmatics (16) and add to existing circumstantial evidence supporting the hypothesis that TARC/CCL17, MDC/CCL22, and IP-10/CXCL10 play a mechanistic role in asthma from static studies (23–25), following bronchial allergen challenge (12, 26) and in animal “models” of asthma (27–29). To our knowledge there is only one previous report of elevated IP-10/CXCL10 expression in the bronchial mucosa of smokers with COPD (30), which is consistent with our present findings. In contrast, the broad similarity in expression of TSLP and chemokines in two diseases of supposedly distinct etiology raises the question whether these mediators are germane to disease pathogenesis or bystanders in a range of bronchial inflammatory processes.

There is currently intense interest in, but limited information on, the cellular sources of TSLP, stimuli for its production, and possible functional roles in airways disease such as asthma and COPD. Our data confirm bronchial epithelial cells and mast cells as potential sources, as well as identifying neutrophils, monocyte/macrophages, and endothelial cells in both diseases. Other reported potential sources include human synovial fibroblasts and airways smooth muscle cells (21, 31). In the laboratory, potential environmental stimuli leading to increased TSLP expression in bronchial epithelial cells include microbial products (probably through engagement of Toll-like receptors), viral infection, and physical injury (22, 32). Our data suggest that cigarette smoking also increases bronchial mucosal expression of TSLP at least at the level of mRNA. Similarly, pulmonary expression of TARC/CCL17 and MDC/CCL22, two chemokines strongly induced in dendritic cells by TSLP, was selectively elevated in an animal model of chronic cigarette smoke exposure (33), consistent with the hypothesis that smoke inhalation is a stimulus to TSLP production. Endogenous cytokines, particularly TNF- α and IL-1 β and particularly acting in synergy with Th2-type cytokines such as IL-4 and IL-13 can induce TSLP in bronchial (22, 32) and skin (34) epithelium. This and the fact that cytokines such as IL-1 β and TNF- α may be induced in the skin by mechanical trauma and skin barrier disruption (35) may partly explain the expression of TSLP in lesional skin in atopic dermatitis (15). It remains to be determined whether TSLP expression is elevated in asthma and COPD as a

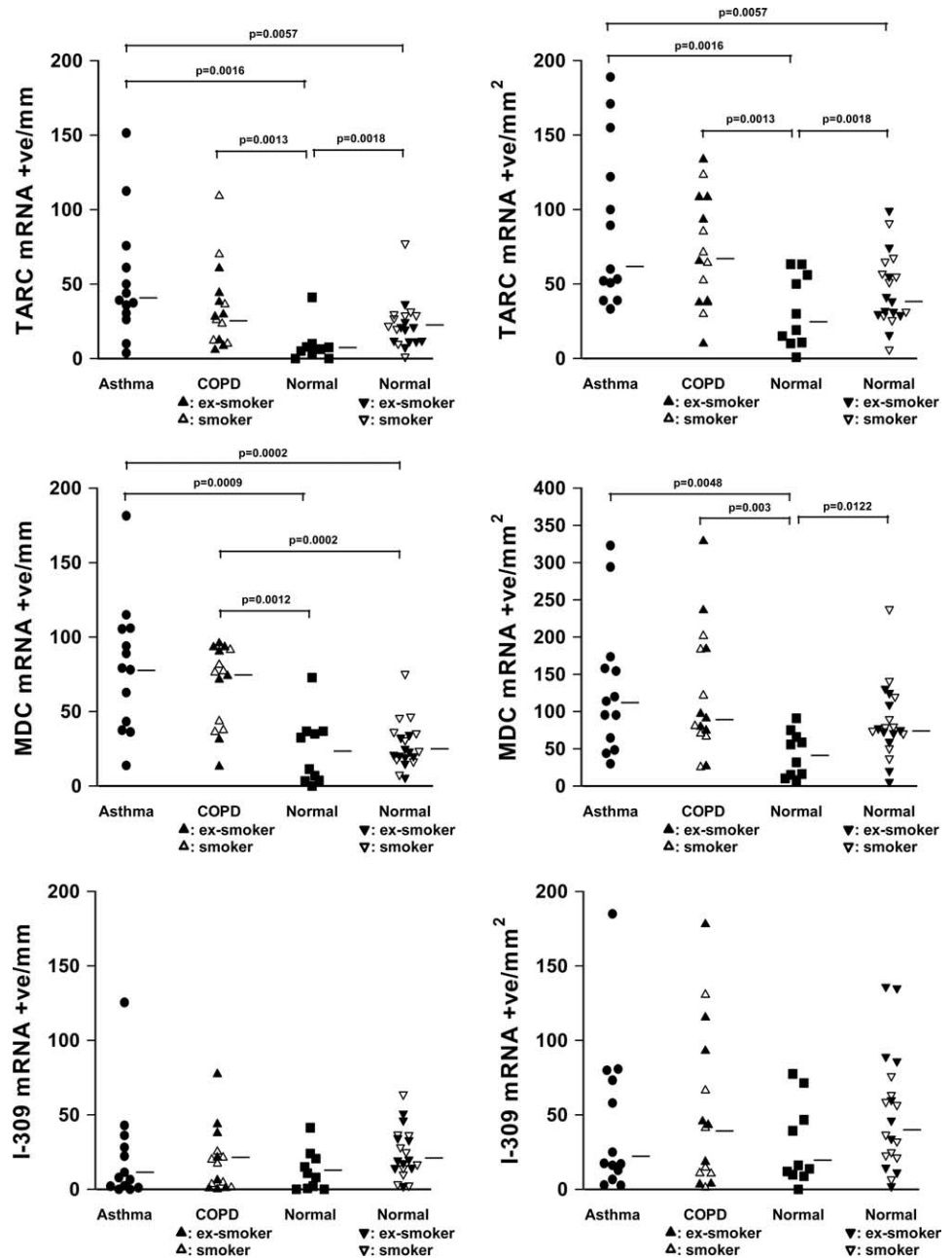


FIGURE 3. Th₂-attracting chemokine (TARC/CCL17, MDC/CCL22, and I-309/CCL1) mRNA⁺ cells in bronchial biopsies. Numbers of cells expressing TARC/CCL17 (*top*), MDC/CCL22 (*middle*), and I-309/CCL1 (*bottom*) mRNA in the epithelium (*left*) and submucosa (*right*) of bronchial biopsies from severe asthma, COPD, and normal controls. Kruskal-Wallis ANOVA (within groups) and Mann-Whitney *U* test (between groups).

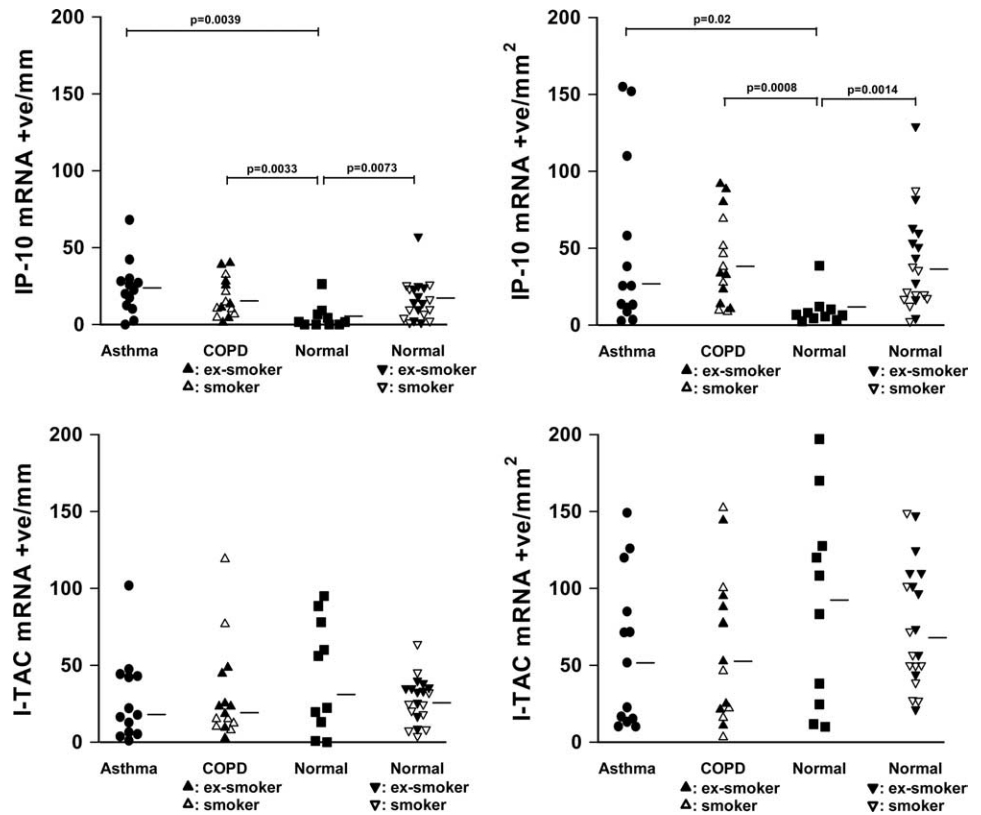
result of environmental stimuli, local production of proinflammatory cytokines or both.

Functionally, TSLP has been shown to increase the production of TARC/CCL17 and MDC/CCL22 by CD11c⁺ dendritic cells in vitro (15) and our data are consistent with the hypothesis that TSLP exerts this effect in the bronchial mucosa in both asthma and COPD in vivo. Our data do not, however, support our original hypothesis that there is clear differential expression of TSLP or chemokines attracting Th1- and Th2- T cells in asthma and COPD. On the contrary, they show very similar expression of these chemokines in both diseases, matched by similar numbers of mucosal cells expressing their ligands CCR4, CCR8, and CXCR3 not only in asthma and COPD but also in the control groups. We conclude that differential local expression of these chemokines is not likely a prominent mechanism whereby populations of functionally distinct T cells are established in the bronchial mucosa. Other investigators have reached similar conclusions (14, 36). This further detracts from

the possibility of a critical, disease-specific role for these mediators. Other chemokines may be important for the recruitment of granulocytes to the airways in both asthma and COPD. For example, we have previously implicated CCR3 ligands such as eotaxin in the recruitment of eosinophils to the asthmatic bronchial mucosa (37). In the present study, increased mucosal expression of IP-10/CXCL10 in the subjects with asthma and smokers with or without COPD could at least partly account for the increased neutrophil infiltration we observed in these subjects.

Another functional effect of TSLP is to prime dendritic cells to promote differentiation of inflammatory CD4⁺, Th2-type T cells (38) through activation with OX40 ligand, and cytotoxic CD8⁺ T cells producing both IL-13 and IFN- γ by additional stimulation through CD40L (39). Although we did not measure cytokine expression in the present study, it is possible to speculate that TSLP may contribute to the elevated expression of Th2-type remodelling cytokines such as IL-4, IL-9 and IL-13 in COPD (40, 41) as well

FIGURE 4. Th₁-attracting chemokine (IP-10/CXCL10, I-TAC/CXCL11) mRNA⁺ cells in bronchial biopsies. Numbers of cells expressing Th1-type chemokine IP-10/CXCL10 (*top*) and I-TAC/CXCL11 (*bottom*) mRNA in the epithelium (*left*) and submucosa (*right*) of bronchial biopsies from severe asthma, COPD, and normal controls. Kruskal-Wallis ANOVA (within groups) and Mann-Whitney *U* test (between groups).



as asthma, and IL-13 mediated cellular cytotoxicity which has been firmly implicated in the pathogenesis of emphysema in animal models (42).

We did not find increased numbers of CD8⁺ T cells in the large airways of patients with COPD as compared with smoking and non-smoking controls as has been reported in some (43, 44) but not all (45, 46) previous studies. These disparate data probably reflect the fact that CD8⁺ T cell infiltration varies with both COPD severity (43, 44, 46)

and the amount and duration of cigarette smoke exposure (44). Otherwise our data, in terms of overall numbers of infiltrating mucosal inflammatory leukocytes in asthma and COPD, are concordant with other comparable studies (47) and also with data suggesting that airways inflammation in patients with or without COPD tends to persist following smoking cessation (48, 49).

As in many other studies of chronic severe asthma and COPD, a proportion of the patients with COPD and all of the asthmatics

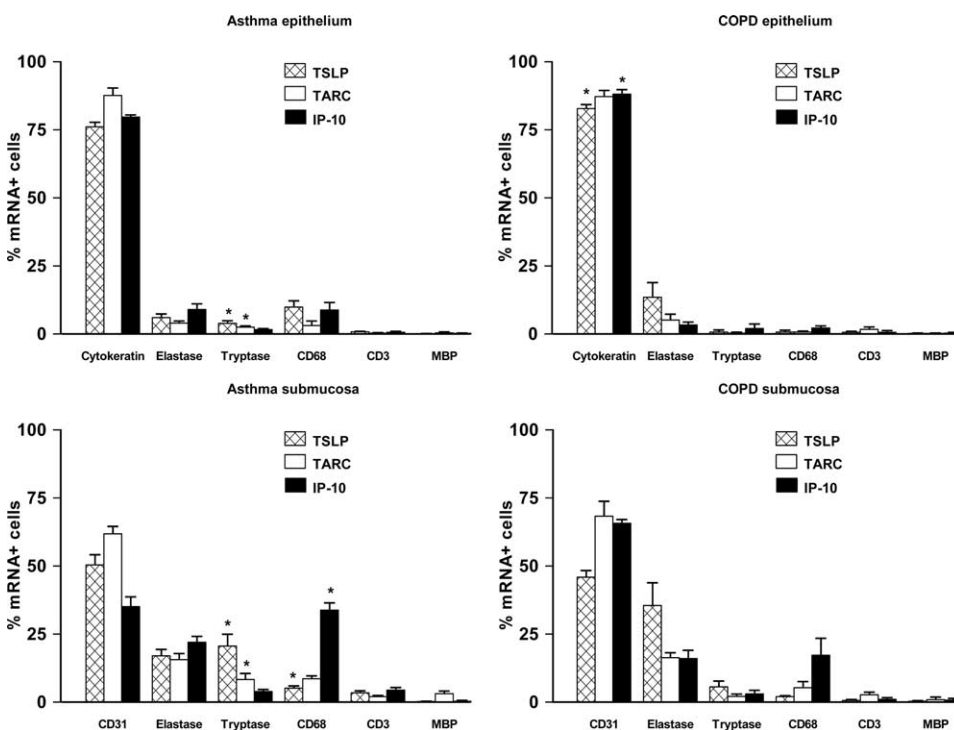


FIGURE 5. Cellular sources of TSLP, TARC/CCL17, and IP-10/CXCL10. Phenotypes of cells expressing mRNA encoding TSLP, TARC/CCL17, and IP-10/CXCL10 in the epithelium (*top*) and the submucosa (*bottom*) of bronchial biopsies from severe asthma (*left*) and COPD (*right*) (smokers and ex-smokers) by sequential IHC/ISH. In each group, *n* = 6. *, *p* < 0.05 (comparing asthma and COPD, Mann-Whitney *U* test). Means and SEM (bars) are shown for clarity.

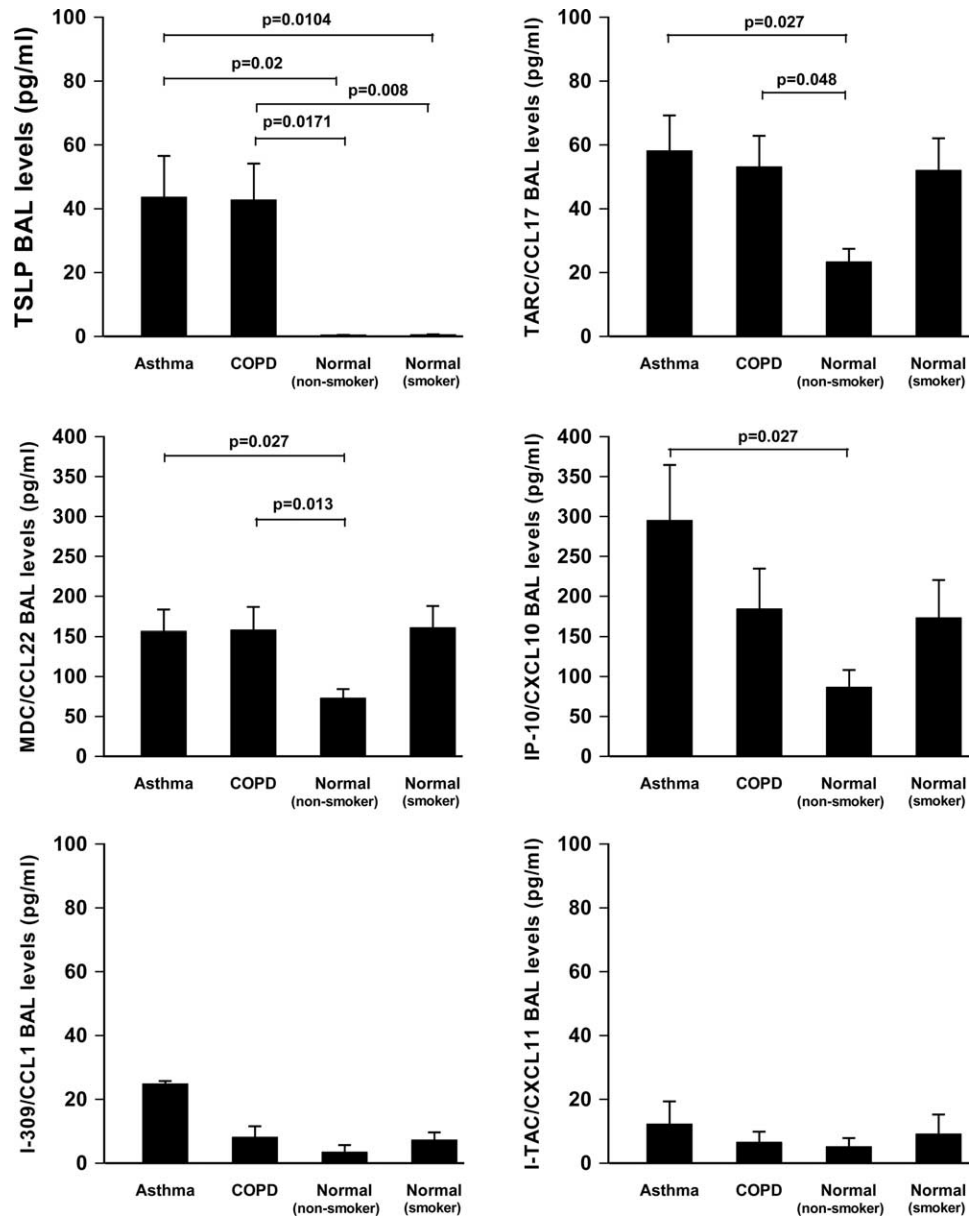


FIGURE 6. Concentrations of TSLP, TARC/CCL17, MDC/CCL22, IP-10/CXCL10, I-309/CCL1, and I-TAC/CXCL11 in BAL fluid from asthmatics ($n = 13$), COPD ($n = 15$), non-smoker controls ($n = 10$) and smoker controls ($n = 20$). Kruskal-Wallis ANOVA (within groups) and Mann-Whitney U test (between groups). Means and SEM (bars) are shown for clarity. The data were normalized to the total protein content of the fluid as described in the text.

were taking inhaled corticosteroids, which it would have been difficult ethically to discontinue for the purposes of this study. Although we cannot rule out a possible effect of inhaled corticosteroids on the expression of any of the mediators measured in the present study, we were not able to detect significant differences in TSLP or chemokine expression in patients taking or not taking inhaled corticosteroid, although the study may have been insufficiently powered to detect such differences. Further studies would be necessary to explore the effects, if any, of corticosteroids on the expression of these mediators.

In conclusion, there is a need for further information as to the range of stimuli for TSLP production in the bronchial mucosa in asthma and COPD and the precise functional consequences of its production. Although the study was not designed or powered to uncover between-subject heterogeneity in bronchial mucosal pathology within the clinical groups studied, overall there would appear to be more similarities than differences between asthma and COPD at least in terms of expression of TSLP and chemokines that selectively attract distinct subsets of functionally differentiated T cells.

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Disclosures

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