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

Sun Ying,* Brian O’Connor,* Jonathan Ratoff,* Qiu Meng,* Cailong Fang,* David Cousins,* Guizhen Zhang,† Shuyan Gu,† Zhongli Gao,† Betty Shamji,‡ Matthew J. Edwards,‡ Tak H. Lee,*, and Chris J. Corrigan*

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.
asthma and COPD is populated by functionally distinct T cells, and that if chemotaxis 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. Enobronchial 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, FEV<sub>1</sub> < 80% of the predicted value, with FEV<sub>1</sub>/forced vital capacity ratio < 0.7), a median 21% (range 16–36%) improvement in FEV<sub>1</sub> following inhaled β<sub>2</sub>-agonist; ICS, inhaled corticosteroid.

Table II. The numbers of inflammatory cells in the epithelium (per mm length of basement membrane) and submucosa (per mm<sup>2</sup> of submucosa) of bronchial biopsies from moderate/severe asthma, COPD, and controls

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

*The data are expressed as the median (range).  
* p < 0.01 (vs COPD and all controls); ** p < 0.05 (vs never-smoker smokers) (Mann-Whitney U test).
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 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 ± 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 0.6, 4.1 to 0.4 mm, respectively, with within-group coefficients of variation from 10.4 to 12.1%. The mean ± 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 ± 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 0.6, 4.1 to 0.4 mm, respectively, with within-group coefficients of variation from 10.4 to 12.1%

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 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. |
|-----------------|-----------------|-----------------|
|                 | 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) |

* The data are expressed as the median (range).

| 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). |
|-----------------|-----------------|-----------------|
|                 | 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) |

* The data are expressed as the median (range).
FIGURE 1. Typical examples of singleISH, sequential IHC/ISH, and double IHC in bronchial biopsies. SingleISH 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 were considerably above the numbers expressing TARC/CCL17 and MDC/CCL22 mRNA (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).

Th\(_1\)-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/
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 ≤ 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-smoking controls, 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 lumens 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-1β 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-1β 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
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.
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...
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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