Identification of Cells Expressing IL-17A and IL-17F in the Lungs of Patients With COPD

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Manuscript received March 25, 2010, accepted August 9, 2010

Funding/Support: This study was funded by an unrestricted research grant from UCB Celltech Ltd. Reproduction of this article is prohibited without written permission from the American College of Chest Physicians (http://www.chestpubs.org/site/misc/reprints.xhtml).

Background

Lymphocytes secrete IL-17A and IL-17F, which enhance innate immune responses. IL-17 expression has not been studied in COPD small airways. The aim of this study was to quantify IL-17A and IL-17F expression in the peripheral lung tissue of patients with COPD compared with control subjects and to identify inflammatory cells that express IL-17.

Methods

IL-17 expression was assessed using immunohistochemistry in peripheral lung tissue (18 patients with COPD and 10 smokers and 10 nonsmokers with normal lung function) and induced sputum (12 patients with COPD and six nonsmokers). Alveolar macrophages from eight patients with COPD, eight smokers, and seven nonsmokers were used for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.

Results

The number of inflammatory cells expressing IL-17A in the small airway subepithelium was higher in patients with COPD than in smokers ($P = .01$) and nonsmokers ($P = .02$). IL-17A expression was higher than IL-17F in this region. IL-17A was expressed by lymphocytes, neutrophils, and macrophages (confirmed by RT-PCR). The expression of IL-17F was greater than IL-17A in epithelial cells and lymphoid follicles, although there were no differences among subject groups.

Conclusions
Our findings indicate different roles for IL-17A and IL-17F in the pathogenesis of COPD. IL-17A plays a role in small airway subepithelial inflammation, whereas IL-17F appears to play a more prominent role within lymphoid follicles.

**Abbreviations**

ANOVA

analysis of variance

RORγt

retinoic acid-related orphan receptor γt

RT-PCR

reverse transcriptase-polymerase chain reaction

Th

T-helper

COPD is characterized by progressive airflow limitation associated with airway inflammation. There is evidence of increased inflammation in both the central and the peripheral airways, involving a range of cell types, including neutrophils, macrophages, and lymphocytes.

It is known that CD8+ T-cell and B-lymphocyte numbers are increased in the airways of patients with COPD compared with control subjects. Furthermore, there are increased numbers of tertiary lymphoid follicles in the lungs of patients with COPD. These follicles are organized structures with a B-cell core, T cells in the periphery, and dendritic cells capable of antigen presentation. Such observations have led to the hypothesis that COPD has an autoimmune component.

IL-17 is produced by inflammatory cells and targets structural cells such as epithelia, fibroblasts, and smooth muscle cells, inducing the production of chemokines that promote neutrophilic inflammation. The IL-17 family comprises six members (IL-17A to IL-17F). IL-17A and IL-17F are produced by a subset of CD4+ lymphocytes known as T-helper (Th) 17 cells. In animal models, Th17 cells contribute to the development of autoimmune disease, although there is evidence for Th17 involvement in human autoimmune diseases. Such observations have led to the hypothesis that COPD has an autoimmune component.

IL-17 is overexpressed in the lungs during acute neutrophilic inflammation and in asthma. It has been reported that IL-17A levels are increased in submucosal biopsy specimens from the large airways of patients with COPD compared with control subjects, but there is no difference for IL-17F. Further important questions regarding IL-17 expression in COPD are (1) whether IL-17 is overexpressed in peripheral lung tissue at the site of small airway disease, (2) whether lymphoid follicles are a site of IL-17 production, and (3) whether immune cells other than lymphocytes produce IL-17 in COPD lungs.

We investigated IL-17A and IL-17F expression in the peripheral lungs and induced sputum of patients with COPD compared with control subjects. The aim of this study was to investigate the expression of IL-17A and IL-17F in the small airways, lymphoid follicles, and immune cell types other than lymphocytes.

**Materials and Methods**

**Study Subjects**

Thirty-eight patients undergoing surgical resection for suspected or confirmed lung cancer were recruited for immunohistochemistry studies (Table 1). A different group of 25 patients undergoing surgery were recruited for gene expression studies using alveolar macrophages (Table 2). Twelve patients with COPD and six nonsmoking control subjects were recruited for induced sputum studies (Table 3). Patients with COPD were diagnosed according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) guidelines based on a smoking history of ≥ 10 pack-years, typical symptoms and airflow obstruction defined as FEV1 < 80% predicted, and FEV1/FVC ratio < 0.7. All patients with COPD were required to be ex-smokers for > 6 months to rule out confounding effects of current smoking. Control groups of normal pulmonary function consisted of ex-smokers with a smoking history of ≥ 10 pack-years and lifelong nonsmokers. All subjects
gave written informed consent. The study was approved by the South Manchester Research Ethics Committee.

### Table 1 -- Immunohistochemistry Patient Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD (n = 18)</th>
<th>Smokers (n = 10)</th>
<th>Nonsmokers (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>72 ± 4.0</td>
<td>67 ± 6.0</td>
<td>69 ± 13.7</td>
</tr>
<tr>
<td>Sex, male (female)</td>
<td>13 (5)</td>
<td>6 (4)</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Smoking history, pack-y</td>
<td>57.0 (12.5–180.0)</td>
<td>30.6 (10.0–46.0)</td>
<td>0</td>
</tr>
<tr>
<td>ICS user, yes (no)</td>
<td>3 (15)</td>
<td>0 (10)</td>
<td>0 (10)</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>1.8 ± 0.5</td>
<td>2.4 ± 0.5</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>63.5 ± 18.5</td>
<td>91.2 ± 12.2</td>
<td>107.7 ± 20.5</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>62.1 ± 9.0</td>
<td>76.5 ± 7.4</td>
<td>74.8 ± 10.4</td>
</tr>
</tbody>
</table>

Data are presented as No., mean ± SD, or mean (range). ICS = inhaled (gluco)corticosteroid.

### Table 2 -- RT-PCR Patient Demographics

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD (n = 8)</th>
<th>Smokers (n = 8)</th>
<th>Nonsmokers (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>72 ± 4.4</td>
<td>69 ± 6.0</td>
<td>65 ± 11.5</td>
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<td>Sex, male (female)</td>
<td>7 (1)</td>
<td>5 (3)</td>
<td>1 (6)</td>
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<tr>
<td>Smoking history, pack-y</td>
<td>46.5 (33.8–59.0)</td>
<td>31.2 (10.0–60.0)</td>
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<td>ICS user, yes (no)</td>
<td>2 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>FEV₁, L</td>
<td>1.9 ± 0.6</td>
<td>2.4 ± 0.5</td>
<td>2.0 ± 0.4</td>
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<tr>
<td>FEV₁, % predicted</td>
<td>67.6 ± 14.2</td>
<td>97.6 ± 12.0</td>
<td>95.5 ± 21.3</td>
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<tr>
<td>FEV₁/FVC, %</td>
<td>59.0 ± 8.5</td>
<td>77.8 ± 3.6</td>
<td>78.8 ± 6.5</td>
</tr>
</tbody>
</table>

Data are presented as No., mean ± SD, or mean (range). RT-PCR = reverse transcriptase-polymerase chain reaction. See Table 1 legend for expansion of the other abbreviation.

### Table 3 -- Sputum Patient Demographics and Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>COPD (n = 12)</th>
<th>Nonsmokers (n = 6)</th>
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<tr>
<td>Age, y</td>
<td>74 ± 7.5</td>
<td>38 ± 6.0</td>
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<tr>
<td>Sex, male (female)</td>
<td>6 (6)</td>
<td>3 (3)</td>
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<tr>
<td>Smoking history, pack-y</td>
<td>55.6 (34.0–123.5)</td>
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</tr>
<tr>
<td>ICS user, yes (no)</td>
<td>8 (4)</td>
<td>0 (0)</td>
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<tr>
<td>FEV₁, L</td>
<td>1.5 ± 0.5</td>
<td>3.8 ± 0.9</td>
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<tr>
<td>FEV₁, % predicted</td>
<td>61.2 ± 13.5</td>
<td>116.6 ± 10.9</td>
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<tr>
<td>FEV₁/FVC, %</td>
<td>47.6 ± 11.3</td>
<td>80.2 ± 8.2</td>
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<tr>
<td>Sputum cell counts</td>
<td></td>
<td></td>
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<tr>
<td>% IL-17A immunoreactive cells</td>
<td>91.9 ± 7.2</td>
<td>80.6 ± 18.7</td>
</tr>
<tr>
<td>% IL-17F immunoreactive cells</td>
<td>21.4 ± 8.3[a]</td>
<td>85.4 ± 7.7</td>
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<tr>
<td>% Neutrophils</td>
<td>78.8 ± 12.6[b]</td>
<td>28.9 ± 10.4</td>
</tr>
<tr>
<td>% Macrophages</td>
<td>16.3 ± 11.5[b]</td>
<td>64.9 ± 11.8</td>
</tr>
<tr>
<td>% Eosinophils</td>
<td>3.3 ± 4.0</td>
<td>1.1 ± 2.0</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>0.4 ± 0.4[c]</td>
<td>1.7 ± 1.2</td>
</tr>
<tr>
<td>Variable</td>
<td>COPD (n = 12)</td>
<td>Nonsmokers (n = 6)</td>
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<tr>
<td>--------------------</td>
<td>---------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>% Epithelial cells</td>
<td>1.1 ± 1.5 [c]</td>
<td>3.4 ± 2.0</td>
</tr>
</tbody>
</table>

Data are presented as No., mean ± SD, or mean (range). See Figure 1 legend for expansion of abbreviation.

a $P = .0001$.

b $P = .0002$.

c $P = .01$ vs nonsmokers.

**Immunohistochemistry**

Tissue blocks were obtained from an area of the lung as far distal to the tumor as possible and then formalin fixed and paraffin embedded. Blocks were labeled using either anti-IL-17A or anti-IL-17F primary antibodies as described in e-Appendix 1. Details of all antibodies used in this study are described in e-Table 1.

**Combined Immunofluorescence and Immunohistochemistry**

Combined immunofluorescence and immunohistochemistry was used to label lung sections with IL-17A and IL-17F with one of the following primary antibodies: mast cell tryptase, neutrophil elastase, major basic protein, CD20, or CD3. See e-Appendix 1 for more details.

**Image Analysis**

Details of the instruments used are provided in e-Appendix 1. The numbers of IL-17A and IL-17F immunoreactive cells were calculated within the epithelial and subepithelial layers of small airways: airways < 2 mm in diameter lacking cartilage or submucosal mucous glands. Positive cells within inflammatory follicles and clusters and within the alveolar walls and spaces also were counted. Cell counts were calculated and standardized to the number of positive cells per square millimeter of the area of interest (cluster, follicle, or subepithelia) or per millimeter length (epithelia or alveolar wall). Alveolar macrophages were quantified as described previously.

**Sputum Induction**

Sputum induction was performed as previously described. To obtain a differential cell count, slides were stained using Rapi-Diff (Biotech Sciences Ltd; Wigan, England) according to supplied instructions. Immunocytochemistry for IL-17A and IL-17F was performed as described in e-Appendix 1.

**Reverse Transcriptase-Polymerase Chain Reaction**

Alveolar macrophages were isolated from resected lung tissue as described previously. Total RNA was isolated and reverse transcriptase-polymerase chain reaction (RT-PCR) performed as described in e-Appendix 1.

**Statistical Analysis**

Data from RT-PCR analysis and immunohistochemistry were nonparametric. Differences in lung tissue among the three patient groups were analyzed using analysis of variance (ANOVA). When ANOVA values of $P < .05$ were obtained, pairwise comparisons between groups were performed using Mann-Whitney $U$ tests. Differences between IL-17A and IL-17F expression in peripheral lung samples were analyzed using ANOVA considering the data from all three groups. Data from quantification of IL-17A and IL-17F immunoreactive cells in induced sputum were parametric, so differences between patients with COPD and nonsmoking control subjects were analyzed using unpaired $t$ tests. Analysis was carried out with GraphPad Instat version 3.06 (GraphPad Software Inc; San Diego, California).

**Results**

**Subepithelial IL-17 Expression**

In peripheral lung tissue specimens, IL-17A expression was observed in the infiltrating cells in the subepithelial regions of almost every small airway examined in all three subject groups. Examples of IL-17A subepithelial expression within lung tissue from patients with COPD, smokers, and nonsmokers are presented in Figures 1A-C.
subepithelial cells per square millimeter was increased in patients with COPD compared with control subjects (ANOVA \( P = .01 \); COPD vs smokers, \( P = .01 \); COPD vs nonsmokers, \( P = .02 \)) (Fig 2C). IL-17F immunoreactive cells were found in fewer small airways, and the number of IL-17F immunoreactive cells in the subepithelial region was lower compared with IL-17A (ANOVA \( P < .0001 \)). There was no difference in IL-17F expression among groups (\( P = .31 \)) (Fig 2D). Examples of IL-17F subepithelial expression within lung tissue from patients with COPD, smokers, and nonsmokers are presented in Figures 1D-E.

Figure 1  A-F, Representative images of IL-17A (A-C) and IL-17F (D-F). Immunohistochemical labeling in small airway epithelium and subepithelium of patients with COPD, smokers, and nonsmokers. IL-17A and IL-17F were detected using 3,3′-diaminobenzidine (DAB) substrate (brown). Arrows show IL-17 immunoreactive cells in the subepithelium. Scale bar = 50 \( \mu \)m (original magnification \( \times \) 200).
Figure 2  Quantitation of IL-17 immunoreactivity in the subepithelium and epithelium of small airways. A-D, The percentage of small airways with IL-17A and IL-17F immunoreactive cells in the subepithelium and the number of IL-17A and IL-17F immunoreactive cells in subepithelium are shown. E-H, The percentage of small airways with IL-17A and IL-17F immunoreactive cells in the epithelium and the number of IL-17A and IL-17F immunoreactive epithelial cells are shown. Peripheral lung tissue from patients with COPD, smokers, and nonsmokers was examined. *P = .01. ‡P = .02.

Dual-label immunohistochemistry and immunofluorescence were performed on a subset of samples in order to descriptively determine which cell types expressed IL-17. Representative images of neutrophil IL-17A and IL-17F expression are shown in Figure 3. IL-17A and IL-17F were expressed by a proportion of neutrophils (Fig 3), mast cells, T cells, and B cells in the subepithelium of small airways (e-Figures 1, 2). IL-17F also was expressed by vascular endothelial cells in the subepithelium. IL-17A and IL-17F expression in eosinophils could not be accurately determined because this cell type was not present in sufficient enough numbers.
Figure 3  Representative images for the dual immunohistochemical and immunofluorescent analysis of IL-17 in small airway subepithelial neutrophils. A and D, NE was detected using an Alexa 568-specific IgG stain (red), and cells were counterstained using 4′,6-diamidino-2-phenylindole (blue). B and E, The tissue then was colabeled using either IL-17A or IL-17F and detected using DAB substrate (brown). C and F, Merged images show IL-17A and NE coexpression and IL-17F and NE coexpression, respectively. Red arrows show neutrophils that coexpress either IL-17A or IL-17F. Scale bar = 50 µm (original magnification × 200). NE = neutrophil elastase. See Figure 1 legend for expansion of the other abbreviation.

**Epithelial IL-17 Expression**

Representative images of small airway epithelial expression of IL-17A and IL-17F within lung tissue of patients with COPD, smokers, and nonsmokers are presented in Figure 1. In peripheral lung tissue specimens, IL-17A expression in epithelial cells was observed in fewer small airways compared with IL-17F (\(P < .0001\)). The number of IL-17F immunoreactive epithelial cells per millimeter were significantly higher compared with IL-17A (ANOVA \(P < .0001\)). There was no difference between patients with COPD and control subjects in the percentage of small airways expressing IL-17A (\(P = .08\)) or in the number of IL-17A immunoreactive cells per millimeter (\(P = .50\)). There was no difference between patients with COPD and control subjects in the percentage of small airways expressing IL-17F (\(P = .06\)) or in the number of IL-17F immunoreactive epithelial cells (\(P = .13\)) (Figs 2E-H). It is possible that IL-17A (or IL-17F) is bound to its receptor expressed by epithelial cells; however, IL-17 receptor expression has been detected at the basolateral level only.\(^{[29]}\) We observed IL-17A and IL-17F expression throughout the cell and not only at the basolateral level, suggesting that epithelial cells produce IL-17.

**Alveolar Wall IL-17 Expression**

Examples of IL-17A and IL-17F expression within peripheral lung tissue specimens from patients with COPD, smokers, and...
nonsmokers are presented in Figures 4A-F. In peripheral lung tissue specimens, IL-17A and IL-17F expression was observed in cells within the alveolar walls of all three patient groups (Figs 4A-F). IL-17A and IL-17F immunoreactivity was observed in pneumocytes (type I and II) and the infiltrating immune cells in all three groups. There were greater numbers of IL-17A immunoreactive cells compared with IL-17F (ANOVA $P < .0001$). The number of IL-17A and IL-17F immunoreactive alveolar wall cells was similar in all three groups ($P = .17$ and $P = .35$, respectively) (Figs 5A, 5B).

**Figure 4** IL-17 immunoreactivity in the alveolar wall and lymphoid follicles of peripheral lung tissue. A-F, Representative images for the immunohistochemical labeling of the alveolar walls for IL-17A and IL-17F. Type 1 and type 2 (red arrows) pneumocytes are shown. IL-17 was detected using DAB substrate (brown). G-L, Representative images for the immunohistochemical labeling of lymphoid follicles for IL-17A and IL-17F. Scale bar = 50 µm (original magnification × 200). See Figure 1 legend for expansion of abbreviation.
IL-17 Expression in Lymphoid Follicles

In peripheral lung tissue specimens, IL-17A immunoreactive cells were found in the periphery of most lymphoid follicles (Figs 4G-I). IL-17F immunoreactive cells were seen throughout the lymphoid follicles, including the B-cell core and T-cell periphery (Figs 4J-L). Consequently, the number of IL-17F immunoreactive cells per square millimeter was higher compared with IL-17A (ANOVA $P < .0001$). There were no differences between patients with COPD and control subjects for the number of IL-17A (Fig 5C) or IL-17F (Fig 5B) immunoreactive cells in lymphoid follicles (ANOVA $P = .44$ and $P = .16$, respectively).

IL-17 Expression in Alveolar Macrophages

In peripheral lung tissue specimens, IL-17A (Figs 6A-C) and IL-17F (Figs 6D-F) expression were observed in alveolar macrophages. There were no differences between patients with COPD and control subjects for the percentage of either IL-17A or IL-17F immunoreactive alveolar macrophages ($P = .83$ and $P = .31$, respectively) (Fig 7). The percentage of IL-17F immunoreactive alveolar macrophages was significantly higher than IL-17A (ANOVA $P < .0001$) (Fig 7).
Figure 6  A–F, Representative images for the immunohistochemical analysis of alveolar macrophages for IL-17A and IL-17F in patients with COPD, smokers, and nonsmokers. IL-17 expressing alveolar macrophages are highlighted. IL-17 was detected using DAB substrate (brown). G and H, Typical negative controls for IL-17A and IL-17F are shown. Scale bar = 50 µm (original magnification × 200). See Figure 1 legend for expansion of abbreviation.

Figure 7  A, B, IL-17 expression in alveolar macrophages. The percentage of IL-17A (A) and IL-17F (B) immunoreactive alveolar macrophages are shown.

To support immunohistochemical findings, mRNA levels in alveolar macrophages from a separate group of patients were assessed. IL-17A and IL-17F expression levels were similar. There were similar levels of IL-17A (ANOVA $P = .18$) and IL-17F (ANOVA $P = .08$) gene expression levels between patients with COPD and control subjects. IL-6 mRNA levels were higher than IL-17A and IL-17F in all patient groups (ANOVA $P < .0001$) (Fig 8).
A-C, IL-17 mRNA levels in alveolar macrophages. The relative expression levels of IL-17A (A), IL-17F (B), and IL-6 (C) are shown; mRNA levels were normalized to a geometric mean of 18S/glyceraldehyde-3-phosphate dehydrogenase/β-actin.

**IL-17 Expression in Sputum Neutrophils**

Induced sputum samples were used to study IL-17 expression in neutrophils. The percentage neutrophil counts was higher in patients with COPD than in nonsmokers (Table 3). Strong IL-17A immunoreactivity (Figs 9B, 9C) was observed in patients with COPD and nonsmokers; morphologic analysis showed that sputum macrophages and neutrophils expressed IL-17A. There was no significant difference ($P = .08$) between the percentage of IL-17A immunoreactive sputum cells in patients with COPD (91.9%) and that in control subjects (80.6%) (Fig 9A).
Figure 9  A, Quantification of IL-17A and IL-17F within sputum samples. Representative images for the immunocytochemical analysis of sputum for IL-17. B-E, Typical images of sputum cells for IL-17A and IL-17E in patients with COPD and nonsmokers are shown. IL-17A and IL-17F were detected using DAB substrate (brown). Cells were counterstained using Mayer hematoxylin (blue). IL-17A+ (red arrow 1) and IL-17F- (red arrow 2) neutrophils are shown. Scale bars = 50 µm (original magnification × 200). See Figure 1 legend for expansion of abbreviation.

IL-17F immunoreactivity (Figs 9D, 9E) also was observed in sputum cells, with significantly lower expression levels ($P = .0001$) in patients with COPD (21.4%) compared with nonsmokers (85.4%). Morphologic analysis showed that IL-17F was expressed mainly by sputum macrophages and that the majority of neutrophils did not express IL-17F.

Discussion

We observed increased IL-17A expression in infiltrating inflammatory cells in the subepithelium of the small airways of patients with COPD compared with control subjects. We also observed that IL-17A expression was greater than IL-17F expression in the subepithelial region and that IL-17A was expressed by inflammatory cells other than lymphocytes, including neutrophils and macrophages. In contrast, at other anatomic locations, including the epithelial region, alveolar walls, and lymphoid follicles, there were no differences between patients with COPD and control subjects for either IL-17A or IL-17F expression. The selective upregulation of IL-17A in the small airway subepithelium of patients with COPD suggests a role for this cytokine in the pathophysiology of small airway disease.

There was no difference in IL-17A expression in the subepithelium between nonsmokers and ex-smokers, suggesting that the burden of long-term cigarette smoke exposure does not upregulate IL-17A expression. In contrast, our data indicate that the development of COPD does upregulate IL-17A expression at this anatomic location.
It is known that increased numbers of inflammatory cells, including neutrophils, macrophages, and lymphocytes infiltrate the subepithelial region of the small airways of patients with COPD, [1] . [30] . [31] We have shown that IL-17A is expressed by all of these cell types in the airways. This cytokine is well-known to be produced by T cells, but we show that pulmonary macrophages and neutrophils also express IL-17A. Previous reports of IL-17A expression in the airways have focused on the large airways and reported increased numbers of IL-17A immunoreactive cells in the bronchial submucosa of patients with COPD [23] and of patients with moderate-to-severe asthma. [21] The physiologic role of IL-17A in the subepithelium may be to orchestrate subepithelial fibrosis through activation of fibroblasts. [20]

IL-17F was expressed by lymphocytes and macrophages but unlike IL-17A, had low expression levels in neutrophils. Furthermore, IL-17F levels were lower in the subepithelium compared with IL-17A, suggesting that IL-17A plays a more significant role in inflammatory processes within the small airway subepithelium. However, IL-17F expression in epithelial cells was far higher than IL-17A in the small airways. Reports that IL-17F induces intracellular adhesion molecule 1 expression in primary bronchial epithelial cells [32] . [33] and that lung-specific overexpression of IL-17F in mice leads to infiltration of lymphocytes and macrophages and mucus production [34] suggest a proinflammatory role for IL-17F within the small airway epithelium. This difference between IL-17A and IL-17F was not observed in the bronchial epithelium from the large airways of patients with COPD. [23]

IL-17A and IL-17F often are coexpressed in CD4+ T cells. [35] It is also known that subsets of Th17 cells may produce only IL-17A or IL-17F, as we observed here. Evidence from knockout mice in different disease models suggest overlapping, but differential functions of IL-17A and IL-17F [34] ; for example, IL-17A but not IL-17F was required to induce experimental autoimmune encephalitis, whereas IL-17F but not IL-17A was required to induce airway neutrophilia after allergen stimulation and severe immunopathology in a colitis model. Th17 development and IL-17 expression are controlled transcriptionally by the retinoic acid-related orphan receptor γt (RORγt), which is regulated by cytokines such as IL-6, transforming growth factor β, IL-21, and IL-23. [36] . [37] . [38] Other transcription factors, such as interferon regulatory factor 4, cooperate with RORγt in the induction of Th17 cells. [39] The differential expression patterns of IL-17A and IL-17F that we observed within the small airways suggest that local cytokines such as transforming growth factor β, IL-6, IL-21, and IL-23 regulate transcription factors such as RORγt and interferon regulatory factor 4 in a cell-type-specific manner that determines the expression of IL-17A and IL-17F. Additionally, a change in local cytokine expression could be responsible for the upregulation of IL-17A that we observed in the subepithelium of patients with COPD.

It has been reported previously that IL-17A is expressed by T cells in the BAL of healthy subjects. [19] To our knowledge, we present here the first evidence that IL-17A and IL-17F are expressed in lymphoid follicles of peripheral lung tissue. In follicles, IL-17A and IL-17F are expressed in different areas; IL-17A immunoreactive cells are scattered around the T-cell periphery, and IL-17F immunoreactive cells are found throughout the T-cell periphery and B-cell core, suggesting a different role for each of these cytokines, with IL-17F associated with B-cell function.

There was no change in the overall expression levels of IL-17A or IL-17F within individual follicles from patients with COPD and control subjects. It has been proposed that COPD has an autoimmune basis, with lymphoid follicles serving as a site for antigen presentation, [5] as the number of lymphoid follicles increases in the lungs of patients with COPD. [1] Increased follicle numbers in patients with COPD suggest increased antigen presentation. Our findings suggest a role for IL-17A and IL-17F in these lymphoid follicles.

We have demonstrated that alveolar macrophages express IL-17A and IL-17F at the level of protein and mRNA. The PCR data importantly show that macrophages are producing IL-17 and are not simply target cells. Similarly, our epithelial immunohistochemistry showing cytoplasmic IL-17 expression indicates production by these cells. IL-17A immunoreactive monocytes and macrophages have previously been seen in patients with inflammatory bowel disease [16] and rheumatoid arthritis. [40] There was no upregulation of IL-17A or IL-17F expression in COPD alveolar macrophages. Our quantification of immunohistochemistry normalized for the number of macrophages, and similarly, PCR analysis normalized for quantity of RNA. However, it is well known that the total number of alveolar macrophages is increased in patients with COPD. [1] . [41] We can infer that the overall burden of IL-17 expression on alveolar macrophages in the lungs of patients with COPD is increased because there are simply more alveolar macrophages in COPD, with the same proportion of these cells expressing IL-17 compared with healthy subjects. Our immunohistochemistry analysis showed IL-17F expression to be greater than IL-17A in alveolar macrophages, although no difference was observed at the mRNA level. This discrepancy between mRNA and protein levels has been reported previously for IL-17 and suggests that posttranscriptional mechanisms are important in determining IL-17 protein levels. [23]

Neutrophil numbers are known to be increased in the airways of patients with COPD [1] ; we observed this pattern in induced sputum. These neutrophils expressed more IL-17A than IL-17F. The decrease in IL-17F expression in sputum cytopsinbs from patients with COPD compared with healthy control subjects was due to the low number of IL-17F immunoreactive neutrophils in sputum. IL-17A expression by neutrophils has been shown previously in mice, [42] . [43] but IL-17F expression in neutrophils has not been reported. These findings suggest a more prominent role for IL-17A in neutrophilic lung diseases because it is expressed by neutrophils to a greater degree than IL-17F.
All the patients in the current study were ex-smokers, ruling out any current effect of cigarette smoking. It certainly would be of interest to evaluate whether current smoking regulates IL-17 expression. The average age of the patients within this study was 70 years, so future studies investigating IL-17 expression within a younger cohort to rule out any possible age-related effects would be interesting. We used peripheral lung tissue from patients undergoing surgery for suspected or confirmed lung cancer, similar to many previous studies of COPD small airways. Any effect of cancer is ruled out or minimized by taking tissue far distant to the tumor and by using control groups.

Our findings indicate different roles for IL-17A and IL-17F in the pathogenesis of COPD; IL-17A plays a role in small airway submucosal inflammation, whereas IL-17F appears to play a more prominent role within lymphoid follicles. Our findings that IL-17A is expressed by immune cell types other than lymphocytes, such as neutrophils and macrophages, show that we should not just think about Th17 cells when considering the role of IL-17 in airway diseases.

Acknowledgments

Author contributions: Dr Eustace: contributed intellectual and technical input to the study design and manuscript preparation.

Dr Smyth: contributed intellectual and technical input to the study design and manuscript preparation.

Ms Mitchell: contributed to the technical work and analysis of the study and review of the manuscript.

Ms Williamson: contributed to the technical work and analysis of the study and review of the manuscript.

Dr Plumb: contributed intellectual and technical input to the study design and manuscript preparation.

Dr Singh: contributed as principal investigator for this study and was involved in all aspects of the writing, reviewing, and revision of the manuscript.

Financial/nonfinancial disclosures: The authors have reported to the CHEST the following conflicts of interest: Dr Singh has received lectures fees, support for conference attendance, advisory board fees, and research grants from a range of pharmaceutical companies, including GlaxoSmithKline, Chiesi Pharmaceuticals, AstraZeneca, CIPLA, Novartis, Forest, MSD, Boehringer Ingelheim, and Allmiral. Drs Eustace, Smyth, and Plumb and Mss Mitchell and Williamson have reported to CHEST that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Other contributions: We thank Helen Edwards, PhD, for her help with performing the RT-PCR and the subsequent data manipulation.

Additional information: The e-Appendix, e-Table, and e-Figures can be found in the Online Supplement at http://chestjournal.chestpubs.org/content/139/5/1089/suppl/DC1.

Web Extra Material

Online Supplement

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