Expression of Epidermal Growth Factors and Their Receptors in the Bronchial Epithelium of Subjects With Chronic Obstructive Pulmonary Disease

Willem I. de Boer, PhD,1 Chi M. Hau,1 Annemarie van Schadewijk, MSc, Jan Stolk, MD, PhD,1 J. Han J.M. van Krieken, MD, PhD,2 and Pieter S. Hiemstra, PhD1

Key Words: Chronic obstructive pulmonary disease; COPD; Epithelium; Epidermal growth factors; Receptors

Abstract

Smoking may affect epithelial repair and differentiation differentially in smokers with and without chronic obstructive pulmonary disease (COPD). We hypothesized that epithelial repair is disturbed in patients with COPD owing to higher expression of epidermal growth factor (EGF)-like factors and/or receptors. We studied epithelial expression of EGF, transforming growth factor α, amphiregulin, heregulin (HRG), betacellulin (BTC), and their receptors, EGFR, HER-2, and HER-3, by immunohistochemical analysis in resected bronchial tissue from 20 subjects with (forced expiratory volume in 1 second [FEV1] <75% of predicted value) and 18 without (FEV1 >85% predicted value) COPD. All subjects underwent surgery for lung cancer. The proportion of intact, damaged, goblet, or squamous metaplastic epithelium was similar in subjects with and without COPD. Regardless of smoking status, HRG expression was higher in intact epithelium of patients with COPD than in those without. Subgroup analysis showed higher EGFR expression in intact epithelium of patients with COPD than in those without. Subgroup analysis showed higher EGFR expression in intact epithelium (1.4 times; P ≤ .04) and higher EGF, BTC, and HRG expression in damaged epithelium (1.4-1.8 times; P ≤ .05) of ex-smokers with COPD compared with ex-smokers without COPD. These data support our hypothesis and suggest that current smoking obscures intrinsically higher expression in COPD.

Chronic obstructive pulmonary disease (COPD) is a major health problem that is the fifth leading cause of death worldwide.1 The main risk factor for developing COPD is cigarette smoking. Cigarette smoke exposure may result in airway epithelial damage, squamous and goblet cell metaplasia, and bronchial epithelial hyperplasia.2,3 In smokers with COPD, some of these epithelial alterations were more pronounced than in smokers without COPD.4-6 In COPD, these structural epithelial changes may be caused directly by cigarette smoke compounds or indirectly via triggering of inflammatory cells like neutrophils to release cytotoxic proteins.

Lam7 and Lannan et al8 reported that cigarette smoke or cigarette smoke condensate induced tracheal and lung epithelial damage and shedding. Takeyama et al9 demonstrated that cigarette smoke exposure induces goblet cell hyperplasia and mucin overproduction. Epithelial damage normally is followed by a repair process. In vivo studies on human and rat lung tissue demonstrated that cigarette smoke induced airway epithelial proliferation.10,11 Willey et al12 showed that cigarette smoke condensate induced a squamous epithelial phenotype in bronchial epithelial cells in vitro.

The function and expression of epidermal growth factors (EGFs) and their receptors has been studied extensively in keratinocytes and more recently in airway epithelial cells.13-16 It was shown that EGF-like growth factors (including EGF, transforming growth factor α [TGF-α], amphiregulin, and neuregulins) and their receptors (EGFR, HER-2 to HER-4) are important proteins that orchestrate the epithelial repair process via induction of epithelial migration, proliferation, differentiation, and extracellular matrix synthesis. However, excessive expression of EGF-like factors or their receptors may lead to squamous metaplasia or epithelial hyperplasia. It was shown that
 bronchial epithelium expresses EGF-like factors and their receptors,\textsuperscript{13,16} and that EGF and HER-3 expression is higher in smokers with chronic bronchitis or mild to moderate COPD than in nonsmokers.\textsuperscript{16,17} In addition, several studies pointed out that mediators, including EGF, cause (trans)activation of EGFR, leading to expression of chemokines and mucins and goblet cell metaplasia, features seen in chronic bronchitis.\textsuperscript{14,18-20} This suggests that EGF and EGFR family members are involved in the development of epithelial changes in COPD and, thus, may contribute to the development of airflow limitation in COPD.

We hypothesized that cigarette smoke alters the airway epithelial repair process by modulating the expression and/or function of EGF and EGFR family members and affects this process differentially in smokers with and without COPD. Little is known about the expression of these factors in COPD. Therefore, we examined expression patterns of EGF, TGF-\(\alpha\), amphiregulin, heregulin (HRG), betacellulin (BTC), EGFR, HER-2 (also known as c-erb-B2, Neu), and HER-3 (also known as c-erb-B3) in airway epithelium from smokers with and without COPD, all of whom underwent resection for lung cancer. Expression was studied by immunohistochemical analysis in areas of epithelial damage and squamous metaplasia and compared with expression in intact epithelium.

### Materials and Methods

#### Antibodies

Mouse monoclonal or rabbit polyclonal antibodies were purchased as follows: mouse anti-BTC (R&D Systems, Minneapolis, MN); mouse anti-EGF (Sigma, Zwijndrecht, the Netherlands); mouse anti-human HRG and rabbit anti-amphiregulin (Neomarkers, Fremont, CA); mouse anti-EGFR (BioGenex, San Ramon, CA); mouse antiphosphorylated EGFR (Transduction Laboratories, San Diego, CA); rabbit anti–HER-2, secondary antibodies, and streptavidin-horseradish peroxidase complex (DAKO, Glostrup, Denmark); and mouse anti–HER-3 (Santa Cruz Biotechnology, Santa Cruz, CA).

#### Subjects

Bronchial tissue specimens of subjects with or without COPD were selected retrospectively from the pathology tissue bank, anonymized, and used as described.\textsuperscript{21,22} Tissue specimens were selected from smokers or ex-smokers who underwent lobectomy or pneumonectomy for lung cancer. Ex-smokers were defined as subjects who stopped smoking for at least 1 year before surgery.

We included 20 subjects (9 current smokers) with COPD (forced expiratory volume in 1 second [FEV\(_1\)] \(\leq 75\%\) of predicted value before bronchodilation; reversibility in FEV\(_1\) \(\leq 12\%\) of the predicted value after 400 \(\mu\)g of inhaled albuterol [salbutamol]) and 18 subjects (9 current smokers, 2 nonsmokers) without COPD (FEV\(_1\) before bronchodilation >84\% of predicted value; CO diffusion \(D_{LCO}\) \(\geq 80\%\); and reversibility in FEV\(_1\) \(\leq 12\%\) of predicted value). The total lung capacities were at normal levels (\(\geq 80\%\) of predicted value). Exclusion criteria were as described.\textsuperscript{21,22}

Macroscopically normal-appearing tissue was obtained as far as possible from the tumor. Frequencies of different non–small cell lung carcinoma types did not differ between subject groups. No microscopic dysplasia or neoplasia was observed. All patients lacked upper respiratory tract infection, did not receive antibiotics perioperatively, or had received glucocorticoids in a period of 3 months before resection. Based on the available information, subjects with COPD could not be subdivided into patients with predominantly chronic bronchitis or emphysema. With regard to the lung function data summarized in Table II, the 2 subject groups differed significantly for only FEV\(_1\). No significant differences were observed for age, total lung capacity, or reversibility. The number of pack years

| Table II | Characteristics of Current Smokers and Ex-Smokers or Nonsmokers Without (Non-COPD) and With COPD *
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<tbody>
<tr>
<td></td>
<td>Sex</td>
<td>Age (y)</td>
<td>No. of PY</td>
<td>FEV(_1)</td>
<td>No. of Patients Using Steroids Perioperatively</td>
<td>No. of Patients</td>
<td></td>
</tr>
<tr>
<td>Non-COPD</td>
<td>Smokers F, 1; M, 8</td>
<td>63.3 ± 3.5 (46-77)</td>
<td>46.7 ± 5.9 (23-70)</td>
<td>95.3 ± 3.7 (86-109)</td>
<td>0</td>
<td>9</td>
<td></td>
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<tr>
<td></td>
<td>Ex-smokers F, 2; M, 7</td>
<td>55.7 ± 4.7 (38-69)</td>
<td>34.5 ± 15.6 (1-110)</td>
<td>98.3 ± 1.2 (93-100)</td>
<td>0</td>
<td>7; nonsmokers, 2</td>
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<td>All F, 3; M, 15</td>
<td>57.7 ± 3.2 (27-77)</td>
<td>41.8 ± 7.0 (1-110)</td>
<td>96.7 ± 1.4 (86-109)</td>
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<td>18</td>
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<td>COPD</td>
<td>Smokers M, 9</td>
<td>60.3 ± 3.3 (45-72)</td>
<td>48.3 ± 3.6 (35-68)</td>
<td>61.2 ± 3.6 (37-75)</td>
<td>1</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ex-smokers F, 1; M, 10</td>
<td>67.7 ± 2.6 (53-78)</td>
<td>47.7 ± 4.7 (20-68)</td>
<td>59.3 ± 2.9 (45-72)</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All F, 1; M, 19</td>
<td>64.4 ± 2.1 (45-78)</td>
<td>47.9 ± 2.9 (20-68)</td>
<td>60.2 ± 2.6 (37-75)</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease; FEV\(_1\), forced expiratory volume in 1 second; PY, pack years.

* Data are given as mean ± SEM (range). The ages given are at the time of surgery. The FEV\(_1\) is the percentage of the predicted value before bronchodilation.
was lower in subjects without COPD, but this difference was no longer observed when the 2 nonsmokers in the group without COPD were excluded from the analysis.

**Immunohistochemical Analysis**

Serial formalin-fixed, paraffin-embedded tissue sections (4 μm) were used for immunohistochemical analysis on amphiregulin, BTC, EGF, HRG, TGF-α, EGFR, HER-2, and HER-3. Immunohistochemical analysis was performed essentially as described.21,22 Briefly, after deparaffinization and rehydration, sections to be stained with anti-EGFR were treated with 0.4% (wt/vol) pepsin. Sections to be stained for amphiregulin, BTC, HRG, HER-2, and HER-3 were pretreated by microwave antigen retrieval in 0.01 mol/L of sodium citrate, pH 6.0. Subsequently, sections were preincubated with 1% (wt/vol) bovine serum albumin. Antigen expression was demonstrated with appropriate dilutions of the primary antibodies in conjugated immunoenzyme assays using a secondary biotin-conjugated antibody, a tertiary complex of streptavidin-biotin conjugated to horseradish peroxidase, and 3,3'-diaminobenzidine as the chromogen. Sections were counterstained with Mayer hematoxylin. Incubation with phosphate-buffered saline plus 1% bovine serum albumin instead of the primary antibody served as a negative control sample and revealed no staining.

Hematoxylin staining was used to assess the extent of epithelial intactness, damage, and squamous metaplasia. This was done by using an ocular micrometer measuring the total length of the different areas along the bronchial ring at a magnification of ×250. Periodic acid–Schiff (PAS) staining was used to assess the extent of goblet cell metaplasia. The PAS+ proportion is given relative to the total measured intact epithelial length. The pseudostratified airway epithelium was considered damaged and/or in the repair phase if 1 or 2 flattened cell layers could be detected without the presence of columnar or ciliated cells. Damaged epithelium with cuboid and disrupted cells was regarded as being mechanically damaged and was not taken into account. Epithelium was considered intact if 2 to 3 cell layers of epithelium containing ciliated cells or goblet cells were present. Squamous metaplastic epithelium was defined by the presence of flattened, nonciliated, superficial epithelial cells.

### Table 2

<table>
<thead>
<tr>
<th>Epithelium or Cells</th>
<th>Non-COPD</th>
<th>COPD</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Current</td>
</tr>
<tr>
<td>Intact</td>
<td>60.5 ± 6.0</td>
<td>58.3 ± 7.2</td>
</tr>
<tr>
<td>Damaged</td>
<td>32.4 ± 6.0</td>
<td>34.1 ± 7.6</td>
</tr>
<tr>
<td>Squamous</td>
<td>8.4 ± 2.4</td>
<td>76 ± 3.1</td>
</tr>
<tr>
<td>Goblet</td>
<td>44.3 ± 6.1</td>
<td>46.7 ± 8.4</td>
</tr>
</tbody>
</table>

COPD, chronic obstructive pulmonary disease.

Data are given as mean ± SEM. The proportion of intact, damaged, and squamous metaplastic epithelium is given as percentage of the total length of measured bronchial epithelium. The proportion of goblet cells is given relative to the total length of intact epithelium.

### Staining and Image Analysis

The expression patterns were evaluated by digital image analysis (DIA) (ligands except BTC) or by a semiquantitative method (BTC, receptors). DIA was performed if a diffuse cytoplasmic staining was seen, whereas semiquantitative analysis was performed if the staining pattern was confined to the cellular membrane (receptors) or if nondiffuse cytoplasmic staining was seen (BTC). Semiquantitative analysis was performed in a blinded manner as described using the following arbitrary visual scale: 0, absence of staining; 1, moderate staining; 2, intense staining; 3, very intense staining. Expression was examined in the entire section. A subset was analyzed twice to assess intraobserver variability (Pearson correlation coefficient \( r = 0.74; P < .05 \)).

DIA was performed using the KS400 v2 digital image acquisition and analysis system (Zeiss, Oberkochen, Germany) as described.21 Depending on the area of the section, 1 to 4 different images of the bronchial epithelium were acquired using low-power magnification. Staining intensity within the epithelium was expressed as the number of pixels per millimeter squared of epithelium. DIA data for staining intensity were expressed as mean density of only the stained epithelial area.

### Statistical Analysis

Immunohistochemical data showed a normal distribution. Data are expressed as mean ± SEM. Significance levels were obtained using the unpaired, 2-tailed Student t test. At a \( P \) value of less than .05, differences were considered statistically significant.

### Results

#### Histologic Features

To assess the status of the airway epithelium, we measured the proportion of intact, damaged, and squamous metaplastic areas and the proportion of goblet cells within the intact epithelium using PAS staining. As shown in Table 2, we observed no differences in the type of epithelium or the epithelial integrity...
between subjects with and without COPD, irrespective of current smoking status.

**EGF Receptors**

Expression of the receptors was assessed semiquantitatively because it was not possible to quantify the observed linear staining pattern (in agreement with the receptors being membrane-bound proteins) using our image analysis system. Expression of EGFR was located in basal and some intermediate cells. Expression of HER-2 and HER-3 was observed in all airway epithelial cells, whereas expression of HER-3 was somewhat higher in the apical epithelial cells compared with the basal cells.

For EGFR and HER-2, expression in damaged epithelium was up to 1.3 times higher than in intact epithelium if all subjects were considered together. Within subject groups, expression of all 3 receptors was 1.5 to 2 times higher in damaged compared with intact epithelium. In squamous metaplastic areas, the expression level was comparable to that in intact epithelium except that most of the metaplastic cells were stained. However, no differences in receptor expression were seen between subjects with or without COPD in either epithelial area (Table 3).

We considered the possibility that current smoking obscures differences in expression between subjects with and without COPD. Therefore, we performed a subgroup analysis comparing ex-smokers and current smokers despite the fact that this resulted in small subgroups of 9 subjects each. This analysis showed for EGFR, but not for HER-2 or HER-3, a significantly higher expression of BTC in damaged epithelium of ex-smokers (but not current smokers) with COPD compared with those without COPD ($P = .05$) (Table 3 and Figure 2). BTC expression was not significantly different between subjects with or without COPD in intact epithelium regardless of smoking status. Subanalysis demonstrated that only ex-smokers with COPD demonstrated a significantly higher expression of BTC in damaged epithelium (1.8 times; $P = .02$) compared with those without COPD. We also noted that in subjects without COPD, BTC expression in damaged epithelium was 1.8 times lower ($P = .01$) in ex-smokers than in current smokers.

Finally, HRG expression was 1.2 times higher ($P = .02$) in intact epithelium of all subjects with COPD compared with those without COPD. This difference was more predominant when only ex-smokers were compared (Table 3 and Figure 2). No difference was noted when only smokers were compared. In damaged epithelium, the expression was approximately 1.4 times higher only in ex-smokers with COPD compared with ex-smokers without COPD ($P = .01$; Table 3 and Figure 2). In contrast, current smokers with COPD showed a trend toward lower HRG expression in damaged epithelium (1.2 times; $P = .09$) compared with those without COPD. The expression of EGF, amphiregulin, BTC, and HRG in squamous metaplastic epithelium was not calculated because squamous metaplasia was observed infrequently.

None of the staining scores correlated with FEV$_1$, neither within groups nor when all subjects were considered together. Excluding data from the 2 nonsmokers did not affect the results.

**EGF-Like Growth Factors**

Immunohistochemical analysis for the ligands revealed that TGF-α, EGF, amphiregulin, HRG, and BTC are expressed by most of the airway epithelial cells (Figure 1). For BTC, we noted that goblet cells did not stain. When data from all subjects were considered together, the expression of EGF, amphiregulin, and BTC was significantly higher in damaged epithelium compared with intact epithelium, regardless of the smoking or disease status (Figure 1; Table 3). In contrast, TGF-α expression was up to 1.5 times higher in intact epithelium.

We next compared the expression of ligands and receptors in intact and damaged epithelium between subjects with and without COPD (Table 3) and assessed the impact of current smoking (Figure 2). For TGF-α and amphiregulin, we did not find significant differences in expression patterns between subjects with or without COPD irrespective of smoking status and the intactness of the epithelium. For EGF, no difference in expression was found in intact epithelium between subjects with or without COPD irrespective of smoking status. Subanalysis showed a 1.6 times higher expression of EGF in damaged epithelium of ex-smokers (but not current smokers) with COPD compared with those without COPD ($P = .05$) (Table 3 and Figure 2). BTC expression was not significantly different between subjects with or without COPD in intact epithelium regardless of smoking status. Subanalysis demonstrated that only ex-smokers with COPD demonstrated a significantly higher expression of BTC in damaged epithelium (1.8 times; $P = .02$) compared with those without COPD. We also noted that in subjects without COPD, BTC expression in damaged epithelium was 1.8 times lower ($P = .01$) in ex-smokers than in current smokers.

When comparing ex-smokers with COPD with those without COPD, expression of EGFR, EGF, HRG, and BTC in selected areas of the airway epithelium was higher in COPD.
Immunostaining for epidermal growth factor receptor (EGFR; A, ×200; B, ×200), heregulin (C, ×200; D, ×200), and betacellulin (E, ×200; F, ×200; and G, ×200) in bronchial epithelium of patients without chronic obstructive pulmonary disease (COPD; A, C, E, and F) or with COPD (B, D, and G). Immunostaining for EGFR and heregulin can be seen in intact epithelium and immunostaining for betacellulin in intact epithelium (E) and damaged epithelium (F and G).
In intact epithelium in ex-smokers with COPD, expression of EGFR was higher, whereas no differences were observed in the expression of its main ligands TGF-α, EGF, and amphiregulin. In damaged epithelium, expression of the receptors did not differ between COPD and non-COPD, but expression of EGFR ligands EGF and BTC was higher in ex-smokers with COPD. Finally, expression of the HER-3 ligand HRG was higher in ex-smokers with COPD in intact and in damaged epithelium. Expression of most of the receptors and ligands was higher in damaged epithelium than in intact epithelium, irrespective of the presence of airflow limitation.

Considered together, these data suggest that EGFR, EGF, BTC, and HRG have a role in epithelial repair and remodeling in COPD. In addition, the higher expression found predominantly in ex-smokers with COPD may point to an inhibitory role of cigarette smoke on the expression of these factors in COPD, obscuring the intrinsically higher expression of these factors.

### Table 3

Expression of Receptors and Ligands in Airway Epithelium of Subjects With and Without COPD

<table>
<thead>
<tr>
<th></th>
<th>Intact Epithelium</th>
<th>Damaged Epithelium</th>
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<tbody>
<tr>
<td></td>
<td>Non-COPD</td>
<td>COPD</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td></td>
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<tr>
<td>EGFR</td>
<td></td>
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</tr>
<tr>
<td>AP</td>
<td>0.3 ± 0.06</td>
<td>0.2 ± 0.07</td>
</tr>
<tr>
<td>BS</td>
<td>1.8 ± 0.06</td>
<td>2.0 ± 0.1</td>
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<tr>
<td>HER-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>1.4 ± 0.07</td>
<td>1.4 ± 0.09</td>
</tr>
<tr>
<td>BS</td>
<td>1.4 ± 0.07</td>
<td>1.4 ± 0.09</td>
</tr>
<tr>
<td>HER-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.16</td>
</tr>
<tr>
<td>BS</td>
<td>0.9 ± 0.1</td>
<td>1.0 ± 0.15</td>
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<tr>
<td><strong>Ligands</strong></td>
<td></td>
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<tr>
<td>TGF-α</td>
<td>78.4 ± 5.8</td>
<td>77.4 ± 8.4</td>
</tr>
<tr>
<td>EGF</td>
<td>43.6 ± 2.5</td>
<td>45.9 ± 3.7</td>
</tr>
<tr>
<td>Heregulin</td>
<td>573 ± 2.4</td>
<td>674 ± 3.7</td>
</tr>
<tr>
<td>Amphiregulin</td>
<td>48.1 ± 3.1</td>
<td>45.9 ± 3.2</td>
</tr>
<tr>
<td>Betacellulin</td>
<td>1.2 ± 0.09</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

AP, apical cells; BS, basal and intermediate cells; COPD, chronic obstructive pulmonary disease; EGF, epidermal growth factor; EGFR, EGF receptor; TGF-α, transforming growth factor α. Data are given as mean (SEM). Expression data for receptors and betacellulin were obtained using a semiquantitative analysis giving data from 0 to 3, whereas for the other ligands, the data are given as the number of pixels per millimeter squared of stained epithelium. All data showed gaussian distribution.

* Data are given as mean (SEM). Expression data for receptors and betacellulin were obtained using a semiquantitative analysis giving data from 0 to 3, whereas for the other ligands, the data are given as the number of pixels per millimeter squared of stained epithelium. All data showed gaussian distribution.

† P = .02; Student t test.

### Figure 1

Differences in expression of receptors and ligands in intact (open bars) vs damaged (closed bars) epithelium (all subjects). Semiquantitative expression data of receptors and betacellulin (A) range from 0 to 3. Data for other ligands (B) are presented as number of pixels per millimeter squared of stained epithelium. Data are given as mean ± SEM. * Statistically significant differences (P < .05; intact vs damaged). AR, amphiregulin; BTC, betacellulin; EGF, epidermal growth factor; EGFR, EGF receptor; HRG, heregulin; TGF-α, transforming growth factor α.
The present study indicates the presence of epithelial remodeling in smokers with and without COPD, including goblet cell metaplasia, squamous metaplasia, and epithelial shedding or damage (Table 2) compared with literature data on healthy nonsmokers.6,24 These features also have been reported in other studies.6,10,24 Our finding that these features of the airway epithelium do not differ between patients with and without COPD, irrespective of smoking status, is in agreement with findings reported by others.

Saetta et al6 did not find differences in the proportion of goblet cells between smokers with chronic bronchitis and airway obstruction and smokers without chronic bronchitis and with normal lung function. However, the proportion of goblet cells was higher in smokers than in healthy nonsmoking individuals. It remains to be resolved why no differences are found in epithelial morphologic features between ex-smokers with and without COPD. With regard to goblet cell metaplasia, several studies pointed out that apart from cigarette smoke, a variety of factors can induce goblet cell metaplasia, including cytokines such as interleukin (IL)-4, IL-9, IL-13, and tumor necrosis factor α; growth factors such as TGF-α and heparin binding EGF; neutrophil-derived products, including hydrogen peroxide, elastase,
and neutrophil defensins; and the β2 agonist albuterol. Other studies indicated that cigarette smoke or its condensate can induce squamous metaplasia in vitro and in vivo in dogs.

Collectively, these data show that cigarette smoke and smoke-induced inflammation may cause the observed changes in epithelial integrity and architecture. Whereas the effect of smoking cessation on epithelial changes has not been reported, pulmonary inflammation is present in patients with COPD who do not currently smoke. In view of the proposed contribution of smoking-induced inflammation in epithelial remodeling in COPD, these data suggest epithelial remodeling may persist after smoking cessation.

Because of the critical involvement of EGFs in epithelial remodeling, we studied the expression of these factors in subjects with COPD. We observed the expression of EGF, TGF-α, amphiregulin, HRG, EGFR, HER-2, and HER-3 proteins in airway epithelium. This finding is in line with data from Polosa et al and O’Donnell et al. However, in contrast with data from Polosa et al, we found BTC protein in bronchial epithelium. In line with our immunohistochemical staining, we also found BTC messenger RNA to be present in various lung epithelial cell lines and in subcultures of primary bronchial epithelial cells (data not shown). Because the epithelial cell cultures were not treated with cigarette smoke, the difference between our findings and the those of Polosa and coworkers cannot be explained by the induction of BTC expression by cigarette smoke.

EGF receptors and their ligands are involved intimately in carcinogenesis. We cannot formally exclude the possibility that EGF receptor ligands from neighboring tumor tissue may have affected our results. However, because the distribution of tumor types was not different between the various subject groups studied, all subgroups would have been affected in a similar manner. Therefore, we conclude that the observed differences reflect differences related to COPD.

In general, the expression of the factors studied was higher in damaged epithelium than in intact epithelium regardless of disease or smoking status. The expression data also were not determined by the smoking habit because they were not related to the number of pack years. Moreover, exclusion of expression data from nonsmokers did not affect the study outcomes.

O’Donnell et al demonstrated higher expression of HER-3, but not of EGFR or HER-2, and higher goblet cell numbers when comparing current smokers with or without COPD with healthy nonsmokers. In line with this observation, we did not observe differences in receptor expression between subjects with or without COPD. However, we observed small but significant differences in ex-smokers, a group that was not included in the study by O’Donnell et al.

Although expression of most ligands and receptors in general was lower in ex-smokers without COPD than in current smokers without COPD, the difference reached statistical significance (P = .01) only in the case of BTC in damaged epithelium. The converse was seen in subjects with COPD, showing significantly higher expression of EGFR in intact epithelium and HRG in damaged epithelium in ex-smokers than in current smokers. This indicates that current smoking and the presence of airflow obstruction independently may increase the expression of growth factors and their receptors, whereas current smoking in the presence of airflow obstruction inhibits the mechanisms leading to increased expression. A possible explanation for the latter activity is an inhibitory effect of cigarette smoke on the expression of mediators involved in the regulation of expression of EGFs and their receptors or by (oxidative) inactivation of these mediators.

There is an apparent discrepancy between the presence of differences in expression of EGF family members and EGFR and the absence of corresponding signs of epithelial remodeling. Possibly limited modulation of expression of selected EGF family members as shown in the present study is insufficient for epithelial remodeling in vivo. Alternatively, epithelial cells may show higher proliferation and turnover in COPD, resulting in epithelium equal to that seen in subjects without COPD. Partial support for this is provided by Demoly et al who found more PCNA-positive cells in metaplastic epithelium in patients with chronic bronchitis than in healthy smokers. However, a recent study did not find differences in proliferating epithelial cells, identified by Ki-67 staining, between subjects with or without COPD.

Our results show higher expression of selected HER proteins and ligands in ex-smokers with COPD. The relevance of this expression pattern for our understanding of the pathogenesis of COPD is unclear, but further insight may be provided by ongoing studies in which we are exploring the effect of pharmacological intervention in COPD on features of epithelial remodeling and EGFR expression.

From the Departments of 1Pulmonology, Leiden University Medical Center, Leiden; and 2Pathology, University Medical Center St Radboud, Nijmegen, the Netherlands.

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Address reprint requests to Dr Hijmstra: Dept of Pulmonology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands.

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References


