Chronic Inflammation Is Associated with an Increased Proportion of Goblet Cells Recovered by Bronchial Lavage*

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To evaluate the possibility that bronchoalveolar lavage could provide sufficient respiratory epithelial cells to quantify changes in epithelial cell types associated with chronic inflammation, we examined the epithelial cells obtained in the first infused (20 ml) aliquots that were processed separately from later aliquots, a process known to enrich for bronchial contents. Epithelial cells, including ciliated cells, goblet cells, and fragments of desquamated epithelium, were easily identified after preparation by cytocentrifugation and staining with a modified Giemsa stain. Quantification of the columnar cell types revealed that those with chronic bronchitis and asymptomatic smokers have increased goblet cells as a percentage of the total columnar epithelial cells (chronic bronchitics 36 ± 2 percent, asymptomatic smokers 22 ± 2 percent) compared with normal subjects (9 ± 1 percent, p < 0.001, ANOVA). Significantly, the goblet cell percentage was strongly correlated with other measures of bronchitis and measures of airflow obstruction such as the bronchitis index, a visually derived score at bronchoscopy of airway inflammation (r = 0.72, p < 0.001), the percent neutrophils in the first infused aliquots (r = 0.44, p < 0.05), and the FEV1 percent (r = -0.74, p < 0.001). Thus, bronchoalveolar lavage is capable of providing sufficient bronchial epithelial cells for analysis, and the changes seen in the spectrum of columnar epithelial cells may reflect important underlying pathologic changes.

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The pathologic findings of chronic bronchitis are characterized by goblet cell metaplasia, smooth muscle hypertrophy, submucosal gland hypertrophy, and airway wall fibrosis and inflammation. A feature that is easily identified in patients with bronchitis and smokers is increased numbers of goblet cells in the peripheral airways compared with those seen in normal subjects. Anatomic and pathologic changes in smokers and patients with bronchitis have been shown to correlate with reduced lung function. However, the relationships between therapeutic interventions, airway abnormality, and lung function have not been possible to evaluate because of the difficulties in serially evaluating airway histologic features.

Assessment of airway abnormality has required autopsy material or excised lungs. Smaller specimens such as those obtained with a bronchoscope might be considered assessable except that several investigators have noted that while pathologic changes are consistent within a bronchus, wide variation is seen in the degree of abnormality between bronchioles of the same lung. Thus, even multiple bronchoscopic bi-

opsy specimens might be inadequate for reproducibly quantifying pathologic abnormalities. Evaluation of expectorated sputum has been shown to provide enough cells for evaluation of cell differentials, but the method requires plugs of sputum free of oral contamination and may be hampered by wide variability in cellular composition within sputum samples.

Bronchoalveolar lavage (BAL) is an effective means of sampling lower respiratory tract cells. By processing small (20 ml) first-infused aliquots separately from later aliquots, BAL can yield fluid enriched for bronchial contents. The technique has been useful in assessing airway inflammation. We hypothesized that BAL with separation of the first infused aliquots would be useful in quantifying changes in epithelial cell numbers associated with chronic bronchitis. Bronchoscopy and BAL were performed in 28 patients with chronic bronchitis with airflow obstruction and, for comparison, in 15 smokers without chronic bronchitis and 33 normal, nonsmoking volunteers. The results suggest that BAL provides adequate samples of bronchial epithelial cells for evaluation and that chronic bronchitis with airflow obstruction is associated with increased recovery of goblet cells.

METHODS

The study population was composed of three groups: 28 subjects with a history of cigarette smoking and chronic bronchitis and airflow obstruction, 15 subjects with a history of cigarette smoking,
but without cough, sputum production (asymptomatic smokers), or airway obstruction, and 33 normal, nonsmoking volunteers. Subjects were selected for inclusion in the chronic bronchitis group if they met the following criteria: (1) cough and sputum on most days of the month for at least six months a year during the previous two years; (2) at least one exacerbation, defined as increased dyspnea associated with a change in quality of sputum, which led the subject to seek medical attention, in each of the previous two years; and (3) FEV\(_1\) less than 76 percent of predicted. The subjects included in this investigation have been previously described.\(^{10}\) To minimize clinical variables, the subjects with chronic bronchitis were selected for fixed airway obstruction. Thus, subjects were excluded if the FEV\(_1\) improved by 15 percent or more after inhalation of albuterol, or if the sputum contained 5 percent or more eosinophils. Furthermore, subjects were excluded for evidence of inflammatory lung diseases other than chronic bronchitis, or if the subject had had an exacerbation within six weeks of entering the study. The smoking patients without chronic bronchitis were included only if they were smoking at least two packs per day at the time of the study and had a FEV\(_1\) \(\geq 85\) percent of predicted. The asymptomatic smokers and normal volunteers had not had an upper respiratory tract infection in the six weeks preceding entry into the study.

Informed consent was obtained from all subjects according to the guidelines of the University of Nebraska Institutional Review Board for the Protection of Human Research Subjects.

Spirometry and single-breath diffusing capacity for carbon monoxide were performed according to ATS standards. The predicted normal values used were those of Knudson and coworkers.\(^{11}\)

To obtain bronchial epithelial cells for analysis, bronchoscopy and BAL were performed as previously described.\(^{12}\) Briefly, airway inflammation was visually assessed by two independent observers prior to BAL by examining each lobe and the lingula; the results were quantitated using a "bronchitis index," a scale that grades for erythema, edema, friability, and secretions.\(^{10,12}\) The index was determined by assigning a score (0 to 3; 0 = normal, 3 = severely abnormal) for each characteristic in all six sites and summing all the scores.

BAL was performed by instilling five 20-ml aliquots of sterile normal saline solution, at room temperature, into each of three lobes. Each aliquot was immediately aspirated to minimize dwell time. The first aliquots from each site, the bronchial samples, were pooled and processed separately from the final four aliquots at each site. Bronchial samples prepared in this manner have been found to be enriched with cells and proteins thought to arise from the airways,\(^{13,14}\) and roentgenographic evidence suggests that the earliest lavage aliquots remain substantially within the airways until aspiration.\(^{15}\)

BAL fluids were processed as previously described.\(^{14}\) After removal of mucus by filtration through nylon mesh followed by centrifugation (400g, 8 min), the bronchial sample cells were resuspended in Hank's balanced salt solution and prepared for cytologic examination by cytocentrifugation (Cytospin; Shandon Southern Instruments, Sewickley, PA) of 100,000 cells. The cells were air dried and stained with a modified Wright-Giemsa stain (Diff-Quik; American Scientific, McGaw Park, IL). To more readily identify goblet cells, samples were also stained with a mucicarmine stain. Cell differentials were determined by counting 200 cells in the cytocentrifugation preparations. Epithelial cells were examined on the same preparations.

Data are reported as mean ± SEM. To detect whether differences among means of the three groups existed, analysis of variance (ANOVA) was used. To determine differences between specific groups, \(t\) tests with the Bonferroni adjustment for multiple tests were used. The correlations between cell counts and smoking history, bronchitis index, FEV\(_1\), and age were calculated using Spearman's correlation coefficient.

**RESULTS**

The clinical characteristics of the three groups have been described previously.\(^{10}\) No complications requiring medical attention resulted from the bronchoscopy and BAL in any of the three groups. The chronic bronchitis patients tended to have more cells recovered in the bronchial sample lavage (6.1 ± 2.2 × 10\(^3\)) than did the asymptomatic smokers (3.6 ± 0.6 × 10\(^3\)) or the normal subjects (3.7 ± 0.5 × 10\(^3\)), but all groups had adequate numbers of cells in the bronchial sample for cytologic examination.

Epithelial cells were easily identified using either the modified Wright-Giemsa or the mucicarmine stains. Columnar cell types were clearly identifiable (Fig 1). Three major categories of columnar cells were identified: ciliated columnar cells, cells that had been ciliated but had degenerated and lost visible cilia (also called "cilicytopththoria,"\(^{16}\) and goblet cells. Ciliated cells typically had a columnar shape with an obvious

![Figure 1. Columnar epithelial cells. Aliquots of bronchial sample cells were prepared by cytocentrifugation and air drying prior to staining with a modified Wright-Giemsa stain (Diff-Quik). Goblet cells (G) and ciliated cells (C) are readily identified (original magnification × 400).](http://journal.publications.chestnet.org/pdffileaccess.ashx?url=/data/journals/chest/21631/ on 04/29/2017)
end plate and cilia. A spectrum of degenerating cells was also seen with the cilia partially removed or absent. However, the presence of the end plate and typical morphologic features usually allowed identification as a ciliated type cell. Goblet cells were identified by their different morphologic features with the modified Wright-Giemsa stain. The mucicarmine stain was used in an attempt to specifically stain the mucus-containing goblet cells. However, the mucicarmine stain was not specific in that some macrophages and cellular debris were also stained. Thus, the mucicarmine stain did not provide any particular advantage over the modified Wright-Giemsa stain in determining cell types.

Fragments of bronchial epithelium were also seen and were noted to demonstrate hyperplasia, squamous metaplasia, and reserve cell hyperplasia as has been previously described.24,35 Metaplastic cells can be loosely joined in a cohesive monolayer.36 We did not attempt to quantify these changes.

To quantify changes in the spectrum of epithelial cells recovered, we focused our attention on the single columnar cells. These cells could be easily grouped into ciliated cells (including those degenerated cells in which the cilia were partially gone or lost) and goblet (secretory) cells. Thus, columnar epithelial cell differentials could be counted (Fig 2). The proportions of goblet cells and ciliated cells were determined by two observers each counting 100 columnar cells. There was good interobserver correlation in the counts ($r = 0.81$). The proportions of goblet cells were significantly different when the means of the three groups were evaluated ($p < 0.001$, ANOVA). Furthermore, when the means of goblet cell proportions were compared between the specific groups, the proportion of goblet cells was elevated in the chronic bronchitis group compared with asymptomatic smokers ($p < 0.01$) and the proportion was elevated in the asymptomatic smokers compared with normal subjects ($p < 0.01$).

To correlate the changes in columnar epithelial cells with other measures of bronchitis and airway inflammation and to determine if the presence of airway neutrophils was associated with changes in columnar cells, we compared the percentages of goblet cells with both the visually derived bronchitis index and the percentage of neutrophils in the bronchial lavage cells. The percentage of goblet cells (as a percentage of columnar epithelial cells) correlated with the bron-
Chronic bronchitis index \( r = 0.72, p<0.001 \) (Fig 3). The percentage of goblet cells (as a percentage of columnar epithelial cells) also correlated with the percentage of bronchial sample neutrophils \( r = 0.44, p<0.001 \) (Fig 4). Additionally, the percentage of goblet cells was correlated with pack years of smoking \( r = 0.44, p<0.05 \), percentage goblet cells compared with pack years in the normal nonsmokers and the chronic bronchitic smokers. Thus, increased recovery of goblet cells is associated with other features of bronchitis suggesting that the increased recovery of goblet cells by lavage is reflective of the underlying abnormality of bronchitis with increased goblet cells present in the epithelium.

To determine if changes in the columnar cells were associated with clinical manifestations such as airflow obstruction, we compared the goblet cell percentage with spirometric variables. To control for variations in age, the FEV\(_1\) as a percentage of predicted was used. The percentage of goblet cells was negatively correlated with the FEV\(_1\) percent \( r = -0.74, p<0.001 \) (Fig 5). Also, in the normal nonsmoker group, there was no correlation of the goblet cell percent with age \( r = 0.17, p>0.05 \). Moreover, the percentage of goblet cells was negatively correlated with other measures of airflow obstruction, the FEV\(_1\)/FVC ratio \( r = -0.49, p<0.001 \), and the FEF25-75 \( r = -0.70, p<0.001 \).

**DISCUSSION**

The results of this study demonstrate that bronchoscopy and BAL can be used to provide samples of bronchial epithelial cells sufficient for analysis. Evaluation of the columnar cell types present in bronchial lavage fluid demonstrated increased proportions of goblet cells in patients with chronic bronchitis suggesting that the types of cells lavaged are representative of the underlying pathologic condition. The proportions of goblet cells correlated positively with other measures of airway inflammation, including the visual bronchitis index and the percentage of bronchial neutrophils. Furthermore, the goblet cell prevalence correlated negatively with measures of airflow obstruction, strongly suggesting that the increased goblet cells reflect important underlying pathologic changes.

There are several lines of evidence that the presence of goblet cell metaplasia could be directly related to decrements in lung function. A number of groups have studied morphometric measurements of lung abnormality with lung function using surgical or autopsy specimens, and some of these studies have also examined lesions of the small airways and the epithelial histologic features.\(^9,10,26,27\) Cosio et al\(^{27}\) derived a pathologic score to evaluate eight individual parameters, including the degree of occlusion of the airway lumen, goblet cell metaplasia, squamous cell metaplasia, mucosal ulcers, inflammation, fibrosis, pigment, and amount of muscle. The individual pathologic parameters were not well correlated with lung function, but the total pathologic scores were associated with abnormalities of lung function. Using similar methodology, Wright et al\(^{10}\) demonstrated that goblet cell metaplasia was correlated with reduced lung function in a series of specimens from patients with mild chronic airflow obstruction. Nagai et al\(^{19}\) studied autopsy specimens from patients with moderate to severe chronic airflow obstruction and also showed that goblet cell metaplasia correlated with the FEV\(_1\) percent. The importance of goblet cell metaplasia is also emphasized by the study of Karpick et al\(^{26}\) who found that goblet cell metaplasia was common in patients dying of respiratory failure. The mechanisms by which goblet cells could contribute to airflow obstruction are several fold. Although the volume of epithelial goblet cells is much less than that of submucosal glands, the secretion of mucus by goblet cells may be relatively important. A small increase in mucus production in the peripheral airways may be functionally important in relation to airway obstruction.\(^{26}\) Increased mucus from goblet cells may alter the surface active layer of bronchioles resulting in instability of the these peripheral airways and airways that would more easily close.\(^{30}\)

There are several reasons to support the idea that the prevalence of BAL goblet cells is a reliable marker of long-term changes associated with chronic bronchitis and airflow obstruction. First, the goblet cell percentage was strikingly correlated with declines in the FEV\(_1\), stronger than other measures of airway inflammation such as BAL neutrophils \( r = -0.74 \) for goblet cells and \(-0.44\) for BAL neutrophils. In this regard, the proportion of recovered epithelial cells that are goblet cells may reflect long-standing changes in epithelial cell populations and be less likely to change during short-term variations in the amount of acute airway inflammation. Second, among the smok-
ders, BAL goblet cells correlated positively with the pack-years of smoking ($r=0.44$) suggesting that BAL goblet cells are indicative of long-term accumulative damage from an irritant like cigarette smoke.

BAL appears to be a more practical method for obtaining and evaluating bronchial epithelial cells than other available methods. Transbronchoscopic biopsies would seem much less practical, incur more risk, and are subject to sampling variability. It has been shown that while pathologic changes are consistent within a single bronchiale, there is significant variability among different bronchioles.6,11 Thus, if random transbronchoscopic biopsy specimens are taken, multiple biopsies and an averaging system would have to be employed to minimize sampling error. BAL avoids this problem because bronchi from several sites can be lavaged and multiple subsegmental bronchi are sampled within each site.10 Expectorated sputum cytologic study may provide bronchial epithelial cell samples. Recent investigations have refocused attention on the cell differentials of sputum and the spectrum of abnormalities found in sputum.13,31 Careful attention to preparation is necessary and as many as 15 percent of normal subjects may not be able to give adequate samples.31 However, sputum samples vary greatly in their cellular composition both between different sputum samples and between different parts of a sputum sample.18 BAL avoids these problems because adequate samples are consistently obtained and by pooling the aliquots from multiple lavage sites, the chance of sampling variation is virtually eliminated.

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