Characteristics of Airway Inflammation and Bronchodilator Reversibility in COPD*

A Potential Guide to Treatment

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Study objectives: The management of stable patients with COPD depends on the severity of symptoms and airflow limitation. Regarding inflammation, corticosteroids are the only medications that are recommended for use, and only under restricted circumstances. Corticosteroids tend to undertreat or overtreat patients with COPD when only clinical manifestations and the findings of simple spirometry are considered. Accordingly, our aim was to survey the characteristics of airway inflammation in stable COPD patients, and to assess the interrelations among inflammatory cells, inflammatory mediators, bronchodilator reversibility, and pulmonary function. Factors related to airway inflammation and bronchodilator reversibility may be important in the management of stable COPD patients.

Methods: A total of 88 stable patients with smoking-related COPD were recruited into the study. All patients were steroid-free, and had been treated with theophylline, oral β2-agonist agents, anticholinergic agents, and possibly mucolytic agents. Bronchodilator tests and sputum induction were performed to evaluate bronchodilator reversibility, and numbers of inflammatory cells and mediators (eg, interleukin [IL]-8, eotaxin, and regulated on activation, normal T cells expressed and secreted [RANTES]).

Results: Thirty-one of 48 patients (64.6%) who had bronchodilator reversibility, and 19 of 40 patients (47.5%) without bronchodilator reversibility had sputum eosinophilia (median, 8.0% and 7.0%, respectively). FEV1 showed a significant inverse correlation with the number of sputum neutrophils. The correlation coefficient for postbronchodilator FEV1 vs the percentage of neutrophils in patients with nonreversible COPD was higher than that in those with reversible COPD. The levels of IL-8 were closely associated with the percentage of neutrophils. The sputum concentrations of IL-8 and albumin were significantly higher in patients with nonreversible COPD than in those with reversible COPD. A significant inverse correlation was found between bronchodilator response (ie, ΔFEV1 and ΔFVC) and prebronchodilator FEV1.

Conclusions: Eosinophilic inflammation may play a substantial role in COPD, while neutrophils and IL-8 may have a great influence on nonreversible obstructive airways. The assessment of airway inflammation and bronchodilator responses can help the selection of specific therapies and the prediction of clinical outcomes for COPD patients. (CHEST 2004; 126:375–381)

Key words: COPD; eosinophils; induced sputum; neutrophils; reversibility

Abbreviations: IL = interleukin; RANTES = regulated on activation, normal T cells expressed and secreted

The diagnosis of COPD is based on a history of exposure to risk factors, and the presence of airflow limitation that is not fully reversible and is associated with an abnormal inflammatory response. The management of stable patients with COPD is essentially dependent on the severity of symptoms and airflow limitation. Therapy with bronchodilator medications is central to the symptomatic management of COPD. Regarding the treatment of inflammation, corticosteroids are the only standard medication, and their use has been suggested only under restricted circumstances, as they tend to undertreat or overtreat patients with COPD when only clinical manifestations and the findings of simple spirometry are considered.

It is well-documented that the majority of inflammatory cells in patients with obstructive airway...
diseases are eosinophils in patients with asthma\textsuperscript{2,3} and neutrophils in patients with COPD.\textsuperscript{4–6} However, it has been proposed that there is a significant degree of overlap between asthma and COPD, which is characterized by the predominance of neutrophils in patients with more severe forms of asthma and by increased counts and activation of eosinophils occurring in stable patients with COPD.\textsuperscript{7–9} Currently, corticosteroids are widely used for treatment and are the most effective anti-inflammatory medications available for the treatment of asthma and COPD. Although corticosteroids can effectively modify eosinophilic airway inflammation in asthma patients, and their use correlates with an improvement in symptoms,\textsuperscript{10} the role for steroids in the treatment of COPD is still controversial.\textsuperscript{11} Some reports\textsuperscript{12,13} have suggested that corticosteroids are effective especially for those cases of COPD with eosinophilic airway inflammation. Thus, the characteristics of inflammatory cells in the airways may become a useful guide to the treatment of COPD.

The airflow limitation in COPD is not fully reversible. It has been reported\textsuperscript{14} that the rate of decline in well-preserved FEV\textsubscript{1} correlates negatively with bronchodilator responses in patients with COPD. The higher the initial reversibility, the longer the survival and the smaller the decline of FEV\textsubscript{1}.\textsuperscript{15} One study\textsuperscript{16} has suggested that bronchodilator response is associated with increased exhaled nitric oxide and sputum eosinophilia, indicating a relationship between inflammation and the reversibility of airflow limitation.

The extent of bronchial reversibility and the pattern of inflammation in the airways of COPD patients certainly influence the treatment options and affect the clinical outcome. The above information is thus required before the commencement of treatment. Accordingly, our objective was to survey the pattern of inflammation in stable patients who do not receive steroids for the treatment of COPD and utilize regular medications. Interrelations among inflammatory cells, mediators, bronchodilator reversibility, and pulmonary function were analyzed.

**Materials and Methods**

**Subjects**

This was a prospective study to investigate airway inflammation and bronchodilator reversibility in stable patients with smoking-related COPD who used medications regularly. Eighty-eight male patients were recruited from our outpatient clinic. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease guidelines\textsuperscript{1} from observing symptoms of progressive shortness of breath, productive cough, and occasional wheezing. All patients were treated with theophylline, oral \(\beta_2\)-agonist agents, anticholinergic agents, and possibly mucolytic agents. Subjects had not taken ingested or inhaled corticosteroids for a minimum of 3 months prior to the commencement of the study. They had a smoking history of > 20 pack-years. All subjects were nonatopic, had no history of rhinitis, eczema, or asthma, and had been free from respiratory tract infections or exacerbations for 4 weeks prior to undergoing the pulmonary function tests and sputum induction. Pulmonary function tests were carried out in the morning followed by sputum induction. The control group included healthy subjects who were nonsmokers, nonatopic, and were not receiving any medications. Airflow limitation was characterized by and FEV\textsubscript{1}/FVC ratio of < 70% and FEV\textsubscript{1} of < 80% of normal predicted values after the inhalation of a bronchodilator. Bronchodilator reversibility was defined as an increase of > 12% predicted and 200 mL, respectively, for either the FEV\textsubscript{1} (or \(\Delta\text{FEV}_{1}\)) or FVC (or \(\Delta\text{FVC}\)) above the prebronchodilator baseline. 30 min after the inhalation of 400 \(\mu\)g salbutamol.\textsuperscript{17} Patients did not receive any medication for 24 h before undergoing the bronchodilator reversibility tests. The ethics committee of the hospital approved the study, and written informed consent was obtained from all of the subjects before the study commenced.

**Sputum Induction and Processing**

Sputum production was induced using 3%, 4%, and then 5% hypertonic saline solution after premedication with inhaled salbutamol (400 \(\mu\)g). The opaque and dense portions of induced sputum were selected. If enough of a sample was obtained (ie, four to five mucus plugs), we stopped the inhalation procedure. Samples were weighed to minimize the dilution effect that may influence the final results and were processed with 0.1% dithiothreitol, as described by Pin et al\textsuperscript{18} The supernatant from the cytospin procedure was stained with May-Grunwald-Giemsa stain, and 400 nonsquamous cells were counted. A sample was considered to be adequate when the percentage of squamous cells was < 20%. The differential cell count was expressed as a percentage of the total cell count. The supernatant of the induced sputum samples was aspirated and frozen at \(-80^\circ\text{C}\) until measurement of mediators.

**Measurement of Interleukin-8, Eotaxin, Regulated on Activation, Normal T Cells Expressed and Secreted, and Albumin**

Levels of interleukin (IL)-8, regulated on activation, normal T cells expressed and secreted (RANTES) [BioSource International; Camarillo, CA], and eotaxin (R&D Systems; Abingdon,
UK) in supernatants were assayed by enzyme-linked immunosorbent assay according to the instructions of the manufacturers. The lower limits of sensitivity were 1 to 3 pg/mL for IL-8, 3 pg/mL for RANTES, and 5 pg/mL for eotaxin. The assay results were corrected by quantified albumin in the supernatants. The albumin in supernatants of the induced sputum was measured. A standard curve with a concentration range of 10 to 2,000 pg/mL was obtained by the dilution of purified human serum albumin in phosphate-buffered saline solution. One milliliter of phosphate-buffered saline solution (blank) or standard/sample was added to an equal amount of bromocresol green, and the reaction was allowed to proceed for 10 min. The absorbance rates were measured spectrophotometrically at 628 nm.

Statistical Analysis

Differences between groups were first analyzed using the Kruskal-Wallis test. Intergroup comparisons were assessed by a nonparametric method using the Mann-Whitney U test. Values of p < 0.05 were considered to be significant. Associations among eosinophils, neutrophils, IL-8, albumin, FEV1, and bronchodilator reversibility were measured using the Spearman rank correlation test.

Results

The characteristics of all study subjects are listed in Table 1. The two groups of COPD patients were similar in age and baseline pulmonary function except for bronchodilator reversibility of FEV1 and FVC.

Inflammatory Cells and Mediators

All patients were able to produce sputum after hypertonic saline solution induction. The results for inflammatory cells and mediators are shown in Table 2. Thirty-one of 48 patients (64.6%; median, 8.0%) who had bronchodilator reversibility had sputum eosinophilia, and 19 of 40 patients (47.5%; median, 7.0%) without bronchodilator reversibility had sputum eosinophilia. The percentage of neutrophils was similar in the reversible and nonreversible COPD groups. The levels of albumin and IL-8 in sputum were significantly higher in the patients with COPD without bronchodilator reversibility than in those with COPD with bronchodilator reversibility. There were no differences between the two COPD groups regarding the levels of eotaxin and RANTES.

Correlations Among Pulmonary Function, Inflammatory Cells, and Mediators

Regardless of bronchodilator reversibility, neutrophils exhibited a significant inverse correlation with prebronchodilator FEV1 percent predicted \((r = -0.37; \ p = 0.001)\) and postbronchodilator FEV1 percent predicted \((r = -0.41; \ p = 0.001)\) and prebronchodilator FEV1 percent predicted \((r = -0.57 and p = 0.001)\) vs \(r = -0.31\) and \(p = 0.035\), respectively. The levels of IL-8 were significantly correlated with the percentage of neutrophils \((r = 0.57)\) and albumin \((r = 0.41; \ p = 0.001)\) in induced sputum. However, the prebronchodilator and postbronchodilator FEV1 percent predicted were not correlated with the levels of IL-8.

Bronchodilator Reversibility, Inflammatory Cells, and Pulmonary Function

The changes in FEV1 after the inhalation of salbutamol were not significantly correlated with the percentages of sputum eosinophils or neutrophils. The correlation between the changes in FVC and the percentages of eosinophils or neutrophils were also not remarkable. There was no correlation between bronchodilator reversibility and the levels of IL-8. A significant inverse correlation was observed between \(\Delta FEV1\) percent predicted and prebronchodilator FEV1 percent predicted \((r = -0.41; \ p = 0.001)\), and also between \(\Delta FVC\) percent predicted and prebronchodilator FEV1 percent predicted \((r = -0.41; \ p = 0.001)\).

Discussion

The results of this study show that a substantial degree of eosinophilic inflammation can be observed...
in COPD, although no study subjects had a history of asthma and allergy, and all had received diagnoses that had been determined according to the Global Initiative for Chronic Obstructive Lung Disease guidelines. Sputum neutrophils, but not eosinophils, in stable COPD patients were significantly negatively correlated with FEV1 percent predicted. These correlations were more robust in COPD patients without bronchodilator reversibility than in those with bronchodilator reversibility. In addition, IL-8 was closely associated with the percentages of neutrophils and albumin in induced sputum, and the levels of IL-8 and albumin were significantly higher in patients with nonreversible COPD than in those with reversible COPD. Neutrophils and IL-8 may be associated with the deterioration of pulmonary function. Thus, identifying the characteristics of airway inflammation by using sputum induction may be of great value in guiding the direction of pharmacotherapy in COPD patients and in helping to predict clinical outcomes.

The inflammatory cells that exist in COPD patients are heterogeneous. Neutrophils, eosinophils, mast cells, and CD8+ lymphocytes have been shown to play important roles in inflammatory processes in COPD patients. However, accord-
ing to the percentage and cell numbers, neutrophils and eosinophils are the two major inflammatory cell types in the airways of COPD patients. Corticosteroids are currently the most popular antiinflammatory medications used in the treatment of obstructive airway diseases, although the effect of corticosteroids in the treatment of COPD remains uncertain. In a 3-month, double-blind, placebo-controlled biopsy study, the use of inhaled corticosteroids did not affect the numbers of neutrophils in COPD patients. This phenomenon also was observed in asthma patients with neutrophilic airway inflammation. On the contrary, corticosteroids are effective in the treatment of COPD patients with eosinophilic airway inflammation, not only reducing eosinophil numbers, but also improving clinical symptoms. The response to corticosteroids in patients in whom COPD has been diagnosed might be greater in a subset of patients presenting with more eosinophils and higher levels of eosinophil cationic protein. Therefore, to categorize a distinct subgroup of COPD patients before the commencement of therapy is crucial.

Early in 1958, COPD was defined as a group of diseases with irreversible obstruction of the airways. Nearly half a century later, COPD turned out to be a not fully reversible airflow limitation that is associated with airway inflammation. Antinflammatory therapy has thus emerged as a very important consideration in the management of COPD. A recent study demonstrated that the response to therapy with oral prednisolone appears not to have been a good predictor of the subsequent decline in FEV₁ and the rate of deterioration in health status in a large group of patients with COPD. Regarding lung function, the FEV₁ in COPD patients who have received inhaled corticosteroid treatment was higher than that in the placebo group by at least 70 mL at each time point in a 3-year study, although there was no statistical significance according to the rates of decline in FEV₁. Similarly, Fauwels et al demonstrated that the median decline in the FEV₁ after the use of a bronchodilator over the 3-year period was 140 mL in patients who had received inhaled budesonide and 180 mL in the placebo group. Moreover, therapy with inhaled corticosteroids improves airway reactivity and respiratory symptoms, and decreases the use of health-care services for respiratory problems. The use of inhaled steroids does not affect the long-term progressive deterioration in FEV₁, but differences in lung function between treatment and placebo groups exist. Could some of these COPD patients represent a normal decline in lung function after an appropriate steroid treatment? What are the characteristics of these responders in terms of airway inflammation? Will they have characteristics similar to those in asthmatic patients? We need to investigate the effects of inhaled corticosteroids in COPD patients, and to determine the differences between the patients who benefit from this treatment and those who do not. Feasible sputum inflammation parameters may help to identify those patients who respond to selective therapy with inhaled corticosteroids from those who do not respond.

Neutrophils were inversely associated with the FEV₁ percent predicted observed in our study. This is in agreement with the results of a previous report. We also found that these associations were more prominent in COPD patients without bronchodilator reversibility. Moreover, the levels of IL-8 and albumin were significantly higher in patients with nonreversible COPD, suggesting that neutrophilic inflammation plays a crucial role in nonreversible obstructive airways. It is difficult to know in stable COPD patients whether IL-8 plays a role as a chemoattractant for neutrophils or as a proinflammatory mediator that is released from neutrophils. However, this mediator was not correlated to FEV₁ percent predicted. IL-8 may act as an indicator of the severity of neutrophilic inflammation rather than as an indicator of pulmonary function decline.

Eosinophilic airway inflammation accounted for a substantial proportion of the COPD cases observed in this study. It is unknown how these eosinophils are recruited into the airways. The chemoattractants for eosinophils, including eotaxin and RANTES, were not elevated in COPD patients compared to healthy control subjects. The mechanism of eosinophil influx into obstructive airways is thus unclear. One report has demonstrated that the degree of eosinophilic inflammation is related to early changes in lung function.

**Figure 3.** Correlation of prebronchodilator FEV₁ percent predicted with the percentage of bronchodilator reversibility of FEV₁.
function and smoking habits. The higher counts of eosinophils in induced sputum is associated with the higher number of pack-years and lower values for the midexpiratory phase of peak expiratory flow.28 The mean number of pack-years of smoking found in this study was about 47 pack-years, which is higher than that reported by Pizzichini et al.29 This may partially explain why the mean percentage of eosinophils in the sputum eosinophilia group is higher than that reported by Pizzichini et al.29 (mean, 5.4%). On the other hand, could some of these patients have asthma (without apparent histories) that is contributing to the high degrees of eosinophilia found in our study? It is possible that some asthmatic patients develop COPD because of long-term exposure to cigarette smoke. This may constitute a major part of the overlap between asthma and COPD. However, it is difficult to differentiate between asthma and COPD only by clinical history, clinical manifestations, and spirometry findings. Thus, investigating the characteristics of airway inflammation and creating a practical guide to treatment may surpass the importance of making a specific diagnosis. A question that arises in this regard is how to define the severity of eosinophilic airway inflammation that may require and benefit from corticosteroid treatment. Further study is needed to address these issues.

The absence of short-term responses to bronchodilators suggests that airflow obstruction may be poorly reversible or fixed, a feature that distinguishes COPD from asthma. It should be noted that many patients with COPD have a bronchodilator response.30 In such patients, there is a clinical and functional improvement after therapy with inhaled corticosteroids that is similar to that observed in asthmatic patients.31 A recent study32 demonstrated that COPD patients who are responsive to bronchodilator therapy have a better clinical outcome (with regard to pulmonary function, dyspnea, and health-related quality of life) than those who are not responsive to bronchodilator therapy after 1 year of treatment with an inhaled bronchodilator. In addition, a higher reversibility of airflow obstruction has been proposed as a predictor of a slower decline in FEV1 and better survival.14,15 Thus, bronchodilator reversibility may be a useful indicator not only for assessing the clinical effect of treatment but also for predicting clinical outcome and survival.

It has been suggested that the reversible component in COPD is due to the modifiability of vagal and sympathetic tone.30 The contribution of airway inflammation to bronchial reversibility is unclear. The changes in FEV1 or FVC after the inhalation of β2-agonist agents were not correlated to concentrations of sputum eosinophils, neutrophils, and IL-8. It seems that the extent of the bronchodilator response in COPD patients was not related to airway inflammation, at least in this study. On the contrary, airway reversibility has been reported33 to be associated with the degree of blood eosinophilia. Gross et al.34 showed that greater bronchodilator responses occur in COPD with prebronchodilator FEV1 values < 55% of predicted and are associated with cholinergic tone that is increased in proportion to the severity of airway obstruction. It is compatible with our finding that bronchodilator reversibility inversely correlated with prebronchodilator FEV1 values, suggesting that a substantial degree of reversibility may exist in patients with severe airway obstruction, which can be relieved by therapy with inhaled bronchodilators.

In summary, through the assessment of the characteristics of airway inflammation by sputum induction and the responses of airways to therapy with bronchodilators, we can provide more specific and useful therapies and can help to predict clinical outcomes for patients with COPD.

References


