Respiratory Medicine (2011) **105**, 1885–1890

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journal homepage: www.elsevier.com/locate/rmed

Measurement of soluble perforin, a marker of CD8$^+$ T lymphocyte activation in epithelial lining fluid

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Received 24 February 2011; accepted 18 June 2011
Available online 7 August 2011

KEYWORDS
COPD (chronic obstructive pulmonary disease);
CD8$^+$ T lymphocyte;
Perforin;
ELF (epithelial lining fluid)

Summary
Background: CD8$^+$ T lymphocytes in the peripheral airways have been suggested to be involved in the pathogenesis of COPD. However, the significance of CD8$^+$ T lymphocyte activation in COPD is not well understood. A biomarker of CD8$^+$ T lymphocyte activation in patients with COPD is required.
Methods: Thirty COPD patients and twenty-one healthy controls (eleven ex-smokers and ten who had never smoked or were light ex-smokers) were included in this study. We separately obtained epithelial lining fluid (ELF) from central and peripheral airways using a bronchoscopic microsampling technique. Levels of perforin in ELF were measured and we examined correlations between its values and patients characteristics including pulmonary function.
Results: Perforin levels in both the central and peripheral airways in COPD patients were significantly higher than those in the healthy control groups. In the healthy control groups, there was no significant difference in perforin levels between central and peripheral airways. However, in COPD patients, perforin levels in peripheral airways were significantly higher than those in central airways. Perforin levels in peripheral airways were significantly correlated with FEV_1 (percent predicted), FEV_1/FVC, and DLco (percent predicted) in COPD patients.
Conclusion: The microsampling technique is safe and useful for separately obtaining ELF from central and peripheral airways. Levels of perforin in ELF from peripheral airways were significantly increased and correlated with the degree of pulmonary dysfunction. Perforin might reflect inflammation involving CD8$^+$ T-lymphocytes. This novel biomarker might enable better understanding of the pathogenesis of COPD.

Abbreviations: COPD, Chronic obstructive pulmonary disease; ELF, epithelial lining fluid; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide; IFN, interferon; TNF, tumor necrosis factor; BALF, bronchoalveolar lavage fluid; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ELISA, enzyme-linked immunosorbent assay.

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doi:10.1016/j.rmed.2011.06.008

Introduction

Chronic obstructive pulmonary disease (COPD) is a major worldwide health problem that has exhibited increasing prevalence and mortality. The prevalence, morbidity, and mortality of COPD vary appreciably across countries, but in general are directly related to the prevalence of cigarette smoking. COPD is characterized by airflow limitation that is not fully reversible, usually progressive, and associated with abnormal inflammatory responses of the lung. Indeed, one important pathological feature of COPD is chronic inflammation characterized by an influx of inflammatory cells in the lumen and wall of the bronchial and bronchiolar airways and parenchyma.[1,2] In this respect, there are strong correlations between lung function, airway wall area, the degree of luminal occlusion, and the degree of inflammatory infiltrates in the airways of patients with COPD.[1] However, several studies using surgically resected lung tissue, autopsy lung specimens, and transbronchial biopsies have indicated that more severe inflammatory and structural changes occur in the small airways of COPD.[1,3] Thus, the airflow limitation in COPD probably occurs as a result of small airways inflammation.

There is accumulating evidence that $CD8^+$ T lymphocytes play an important role in the initiation and progression of COPD. In particular, a previous study reported that the number of $CD8^+$ T lymphocytes in COPD lungs was directly related to the degree of airflow limitation.[4] Moreover, a close correlation was found between emphysema assessed by CT and the number of tissue $CD8^+$ T lymphocytes in the lungs.[5] These findings suggest the direct pathophysiological importance of these cells in the decline of lung function in COPD. The presumed role of $CD8^+$ T lymphocytes was suggested by histopathologic studies that found associations between disease severity and the extent of $CD8^+$ T lymphocyte infiltrates within airways of patients with COPD. Since these previous methods are highly invasive, a less invasive biomarker of $CD8^+$ T lymphocyte activation in patients with COPD is required for wide used in clinical investigations.

$CD8^+$ T lymphocytes are believed to lead to chronic inflammation by releasing various inflammatory mediators such as interferon (IFN)-γ, tumor necrosis factor (TNF)-α, as well as perforin.[6] Perforin is about 70kD protein and stored in secretory granules of $CD8^+$ T lymphocytes as a monomer. Perforin is released near target cells and it polymerizes to form transmembrane pores in those cells in the presence of Ca^{2+}. Then granzyme which also stored in secretory granules of $CD8^+$ T lymphocytes enters target cells and leads to apoptosis. It was previously reported that the percentages of T-cells expressing intracellular perforin in bronchoalveolar lavage fluid (BALF) in both former and current smokers with COPD were increased compared to those in individuals who had never smoked.[7] Chrysofakis et al. also reported that perforin expression by $CD8^+$ lymphocytes in induced sputum was significantly higher in smokers with COPD than in those without COPD and healthy non-smokers.[8] These findings suggest that perforin expression may be enhanced in airways of COPD. However, no studies have evaluated the soluble form of perforin in the airways of patients with COPD. We have already established a method of measurement of biochemical constituents in epithelial lining fluid (ELF) samples separately obtained from the central or peripheral airways using a bronchoscopic microsampling technique.[9] Using this method, we attempted to measure the concentrations of the soluble form of perforin in ELF in central or peripheral airways separately, and determined whether its levels were correlated with results of pulmonary function in patients with COPD.

Methods

Subjects

Thirty COPD patients and twenty-one control subjects from the outpatient clinic of our hospital were enrolled in this study. They underwent bronchoscopy to identify the cause of small peripheral nodules. Subjects who agreed to undergo ELF sampling were randomly selected for inclusion in this study. All COPD patients were ex-smokers, and satisfied the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria for the diagnosis of COPD. They had been free of acute upper respiratory tract infections, and none had received inhaled bronchodilators or corticosteroids. Control subjects were divided into two groups based on smoking history (eleven subjects were ex-smokers and ten who had never smoked or were light ex-smokers with less than a five-pack-year history). In this study, we defined the latter group as non-smokers. All ex-smokers had quit smoking at least one year before entering this study. Pulmonary function tests including the diffusing capacity of the lung for carbon monoxide (DLco) were performed in all subjects. In all study subjects, chest computed tomographic scans revealed no abnormal diffuse interstitial infiltrates, and results of arterial blood gas analyses were normal. All subjects gave written informed consent for participation in the study, which was approved by the Ethics Committee of Osaka City University.

Bronchoscopic microsampling technique

ELF was obtained using a previously described bronchoscopic microsampling technique.[9] In brief, after premedication of the subject with atropine and pentazocine, and administration of local anesthesia with aerosolized lidocaine hydrochloride, a flexible fiberoptic bronchoscope (BF-240, Olympus, Tokyo, Japan) was inserted in the trachea and, after flushing with air to minimize contamination of the samples, advanced into the target bronchus. Subsequently, a microsampling probe (BC-402C, Olympus, Tokyo, Japan) was then inserted through the channel of the bronchoscope. The probe consists of a 2.5 mm outer-diameter polyethylene sheath and a 1.9 mm inner polyester fiber rod probe attached to a stainless steel guide wire. Bronchial microsampling was performed in all subjects from a second bronchus (central airway sample). Next, a thin flexible fiberoptic bronchoscope (BF-P260F, Olympus, Tokyo, Japan) was inserted into the lung, and the microsampling probe (BC-401C, Olympus, Tokyo, Japan) was then inserted through the channel of the bronchoscope. This probe consists of a 1.8 mm outer-diameter polyethylene sheath and a 1.1 mm inner polyester fiber