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High levels of interleukin-6 in the exhaled breath condensate of patients with COPD

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Summary *Background*: Chronic obstructive pulmonary disease (COPD) is characterised by chronic inflammation of the respiratory tract.

Methods: We investigated the presence of interleukin-6 (IL-6: a cytokine secreted by monocytes/macrophages, T cells, B cells, fibroblasts, bone marrow stromal cells, keratinocytes and endothelial cells) in the exhaled breath condensate of 16 ex-smokers with moderate COPD, 12 healthy non-smokers. IL-6 was measured by means of a specific enzyme immunoassay.

Results: IL-6 levels were detectable in all of the subjects, but were higher in the COPD patients (8.0 ± 0.1 pg/ml; $P < 0.0001$) than in the healthy non-smokers (4.9 ± 0.2 pg/ml) with a correlation in this group between age and IL-6 levels ($r = 0.597$; $P < 0.05$).

Conclusions: The increased IL-6 levels in exhaled breath condensate may reflect airway inflammation in patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by the presence of airflow obstruction due to chronic bronchitis or emphysema, which usually occur together,[1] and causes the progressive destruction of the alveolar walls.

Previous studies have shown that increased interleukin-6 (IL-6) levels and inflammation may be associated with the progression of COPD.[2] IL-6 is produced in the lung by interstitial fibroblasts, alveolar macrophages, and large-vessel and bronchial epithelial cells. IL-6 levels are high in chronic inflammatory conditions of the lung, such as those due to allogeneic transplantation, bleomycin-induced fibrosis and a variety of human interstitial lung diseases.[3] High levels of IL-6 have been found in the induced sputum of patients with COPD, particularly during exacerbation.[4]

Exhaled condensate may contain molecules coming from the mouth, tracheobronchial system and alveoli, but their proportional contribution is still unclear. It is assumed that airway surface liquid becomes aerosolised during turbulent airflow and that the content of the condensate reflects the composition of airway surface liquid, although large molecules may not aerosolise as efficiently as small and soluble ones.[5]

A strong correlation between the levels of CO_2 and O_2 in exhaled fluid and exhaled breath suggests that aerosol particles exhaled in human breath

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Table 1 Physical characteristics and pulmonary function of control subjects and COPD patients.

Control subjects	Sex	Age(yr.)	Medication*	FEV_1 l (% pred.†)
	M	70	No	3.18(89)
	M	75	No	2.96(98)
	F	57	No	2.09(101)
	F	60	No	2.02(86)
	M	42	No	4.56(90)
	F	55	No	2.57(91)
	M	54	No	2.53(98)
	M	52	No	2.37(88)
	F	75	No	2.87(88)
	F	47	No	2.97(108)
	M	45	No	3.96(100)
	M	52	No	2.5(83)
Mean ± SD		57.3 ± 11.1		2.9 ± 0.7 (93.3 ± 7.5)
COPD subjects				
	M	52	A	1.5(41)
	F	68	B	1.36(48)
	M	70	A	1.23(49)
	M	53	A	1.4(35)
	M	65	A	1.47(44)
	M	72	A	1.35(47)
	M	54	A	1.2(35)
	F	60	A	0.88(38)
	M	74	A	0.9(39)
	M	70	A	1.3(47)
	M	68	A	0.9(35)
	F	57	B	1.1(45)
	M	75	A	1.05(35)
	F	49	A	1.27(43)
	M	75	A	1.05(36)
	F	60	A	0.95(40)
Mean ± SD		63.9 ± 8.9		1.2 ± 0.2(41.1 ± 5.1)

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; data are expressed as mean values ± SD.
*A, inhaled B2 sympathomimetic agent; B, inhaled anticholinergic agent.
†% predicted of European Respiratory Society [7].

reflect the composition of the bronchoalveolar extracellular lining fluid.[6]

The aim of this study was to investigate whether IL-6 can be detected in the breath condensate of COPD patients and to compare its concentration with that in healthy subjects.

Materials and methods

The study involved 16 patients with moderate COPD, and 12 age-matched healthy controls. COPD was diagnosed according to the Guidelines of the British Thoracic Society.[8] All of the patients had been clinically stable for a least 8 weeks. They were all ex-smokers who had given up at least 6 months before the study.

The control subjects had no pulmonary disorders and had shown no signs of upper or lower respiratory tract infection during the previous 8 weeks. All were lifelong non-smokers (Table 1).

Exhaled breath condensate

The breath condensate samples were collected using a specially designed condensing chamber (Ecoscreen; Jaeger, Hoechberg, Germany) as previously described.[9]

IL-6 assay

IL-6 concentrations in the breath condensate were measured using a specific enzyme immunoassay kit (EIA) (Cayman Chemical, Ann Arbor, USA). The

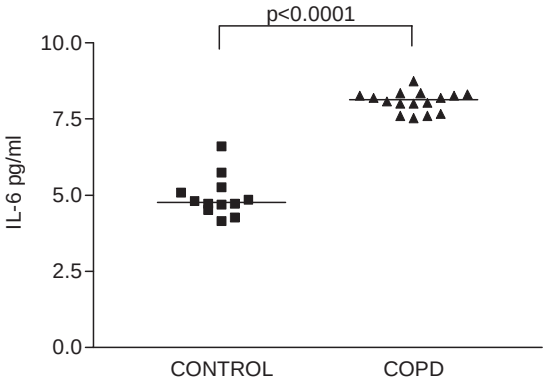

Figure 1 Exhaled breath condensate levels of IL-6 in COPD patients and healthy subjects.

assay was directly validated by means of gas chromatography/mass spectrometry. The intra-assay and inter-assay variability were 10% or less. The detection limit of the assay was 1.5 pg/ml after a 2-h development period. The reproducibility of repeat IL-6 measurements was assessed by the Bland and Altman method and the coefficient of variation.[10]

Statistical analysis

Unpaired *t*-test were used to compare the two groups, and correlations between variables were performed using Spearman's rank correlation test, $P<0.05$ being considered significant.

Results

IL-6 was detectable in the breath condensate of all the healthy non-smokers (4.9 ± 0.1 pg/ml), but was significantly higher in the COPD patients (8.0 ± 0.2 pg/ml; $P<0.0001$) (Fig. 1).

IL-6 levels tended to increase with age in the control group ($r=0.597$; $P<0.05$) but not in the COPD group. No correlation could be found between IL-6 and lung function in either group.

Discussion

This is the first study to examine IL-6 levels, in the exhaled breath condensate of patients with COPD, which were found to be higher than those of the healthy controls.

Increased serum IL-6 levels have been reported in patients with interstitial lung disease and pleural effusion, collagen-vascular disease, inflammatory bowel disease, infectious diseases, and benign or malignant hematological diseases, and it has been suggested that circulating and local levels of IL-6 may reflect disease activity and be useful in evaluating prognosis.[11]

Park et al. found increased IL-6 levels in the BAL fluid of patients with non-specific interstitial pneumonia/fibrosis and in some patients with interstitial pneumonia.[3] Dowlati et al. have reported increased levels of IL-6 in the serum and BAL fluid of patients with lung cancer.[12] A recent study by Bhowmik et al.[4] found increased IL-6 and IL-8 levels in the sputum of COPD patients with frequent exacerbations.

These studies show that inflammatory markers can be detected in the respiratory tract using invasive techniques such as bronchoaleolar lavage or less invasive techniques such as induced sputum. However, these procedures cannot be repeated within a short period of time because they may induce an inflammatory response themselves and are relatively unacceptable for the patients.

Exhaled breath condensate is totally non-invasive and highly acceptable to patients. The collection procedure has no effect on airway function or inflammation, and there is growing evidence that abnormalities in condensate composition may reflect biochemical changes in airway lining fluid.[5] This method has been successfully used in previous studies to investigate several inflammatory markers in COPD and asthma patients such as hydrogen peroxide,[9] isoprostanes,[13] and prostanoids.

The measurement of IL-6 in exhaled air can therefore provide a useful, sensitive, and non-invasive means of studying inflammatory markers. The small increase of IL-6 levels with age in healthy subjects need to be corroborated by studies in a larger population.

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