IL-6 and IL-13 in Induced Sputum of COPD and Asthma Patients: Correlation with Respiratory Tests

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For editorial comment see p. 98

Key Words

Induced sputum • Asthma • Chronic obstructive pulmonary disease • Interleukin-13 • Interleukin-6 • Respiratory disorders

Abstract

Background: IL-6 is strongly implicated in the development of chronic obstructive pulmonary disease (COPD). IL-13 is the well-documented central mediator in allergic asthma. IL-6 is attributed to the proinflammatory activities in COPD as well as asthma. In COPD patients exacerbation is increased by serum IL-6. The association of IL-13 as well as IL-6 with the impaired respiratory function of asthma patients remains controversial. **Objectives:** The aim of this study was to compare the concentration of IL-6 and IL-13 in the induced sputum of asthma and COPD patients, and to assess the possible association of these cytokines with the impairment of lung function. **Methods:** Twenty-six subjects with COPD and 18 subjects with asthma were enrolled in this study. IL-6 and IL-13 levels were measured in induced sputum by ELISA and correlated with the results of respiratory tests. **Results:** The induced sputum of COPD patients had a significantly higher IL-6 level than the sputum of asthma subjects while no significant differences were found in the levels of IL-13. There

was a statistically significant negative correlation between IL-6 level and FEV₁ or FEV₁/FVC in asthma patients ($r = -0.59$ and -0.54 , respectively) and a negative correlation that did not reach statistical significance between IL-6 level and FEV₁, FEV₁% or FVC in COPD subjects ($r = -0.30$, -0.30 and -0.38 , respectively). There was no relationship between concentrations of IL-13 and impaired respiratory function. **Conclusions:** Our results confirmed that IL-6, but not of IL-13, is associated with respiratory disorders in both asthma and COPD patients.

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Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are both inflammatory diseases with similar clinical symptoms [1, 2]. The reversible postbronchodilatory airway obstruction in asthma as opposed to COPD, which is characterized by the progress of airflow limitation that is not fully reversible, is crucial to the differential diagnosis of these diseases. The distinctions between asthma and COPD include reflected differences in the remodeling process and patterns of inflammatory cells and cytokines, as well as the predominant anatomic site

at which these alterations occur [3]. Several studies have recently been conducted to obtain markers able to help distinguish asthma and COPD [2, 4–8]. The results of these studies are inconclusive, but when it comes to cytokines it seems to be well documented that IL-6, a pleiotropic proinflammatory cytokine, plays an important role in the pathogenesis of COPD. Elevated levels of IL-6 in the serum as well as the sputum of COPD patients are associated with impaired lung function [9–12], while IL-13 is a recognized central mediator in allergic asthma [13]. Elevated levels of IL-13 have been found in the sputum/induced sputum of asthma patients compared to control subjects or patients with eosinophilic bronchitis [7, 14]. However, recent studies do not support a role of IL-13 in COPD [6]. The aim of the present study was to compare the concentration of IL-6 and IL-13 in the induced sputum of asthma and COPD patients, and to assess the possible association of these cytokines with impairment of lung function.

Material and Methods

Subjects

Twenty-six subjects with COPD and 18 subjects with asthma were enrolled in the study. Asthma was defined on the basis of clinical symptoms according to GINA [15]. The asthma patients showed airway reversibility (bronchodilator response of >12% and 200 ml increase of FEV₁). All the patients were in a stable stage of the disease and none of the subjects with asthma or COPD had been treated with oral corticosteroids for at least 4 weeks before recruitment. All the patients with asthma were under specialist control. Seven out of 18 asthma patients were treated by a low dose of inhaled corticosteroids with a combination of β_2 -agonists (twice per day); 8 patients were treated by a moderate dose of corticosteroids with combination of long-acting β_2 -agonists, and 3 patients were treated by short-acting β_2 -agonists on demand. Atopic status was assessed through skin prick testing by a panel of fifteen aeroallergens. COPD was diagnosed using GOLD criteria [16]. The severity of disease was assessed using postbronchodilatory spirometric classification: mild in 5 patients, moderate in 14, severe in 4 and very severe in 1. Respiratory function measurements were performed according to the American Thoracic Society standards [17] using an ultrasonic nebulizer ULTRA-NEB™ 2000 (Devilbiss).

The Ethics Committee of the Medical University of Warsaw approved the study, and informed written consent from each study subject was obtained.

Sputum Induction

Pulmonary function tests were performed prior to sputum induction. The patients received 200 μ g of salbutamol before the induction. After the postbronchodilator spirometry they inhaled sterile hypertonic saline (NaCl) at increasing concentrations (3, 4 and 5% solutions) at room temperature via an ultrasonic nebulizer. The duration of each inhalation was 5 min and the induction

was stopped after expectoration of an adequate amount of sputum (2 ml). After each inhalation a spirometry was performed in order to detect a possible FEV₁ decrease. The whole procedure was stopped whenever a 20% FEV₁ decline was observed. In the most cases the decrease of FEV₁ did not exceed 10%.

Sputum Processing

Induced sputum was analyzed immediately on receipt as previously described [18, 19]. The volume of the sputum was measured and the plugs were selected and weighed. A freshly prepared 0.1% solution of dithiothreitol (DTT, Sigma Aldrich Co., St. Louis, Mo., USA) was added in a volume equal to double the weight of the sputum, and the mixture was vortexed for 15 min. Following this a double volume of phosphate-buffered saline (PBS) was added and the mixture was vortexed briefly. After filtration through two layers of a sterile gauze the sputum was centrifuged for 10 min at 800 g. Supernatants were collected and stored at –70°C for interleukin measurements. The selection criteria for induced sputum was as follows: a minimum of 2 ml of expectoration; less than 50% squamous epithelial cells, and more than 200 nonepithelial cells on one slide.

Cytokine Analysis

We performed cytokine measurement only in samples which fulfilled the criteria for induced sputum. The samples were thawed only once for interleukin measurements. IL-6 and IL-13 were measured by ELISA (R & D Systems). The low limits of detection were 0.7 pg/l for IL-6 and 32 pg/l for IL-13. To account for any possible effects of DTT on the cytokines during sputum processing, cytokine standards were reconstituted in PBS-DTT dilution buffer [20].

Statistical Analysis

Results are given as medians (interquartile range) and means. Statistical analysis was performed using Statistical version 9 software. Differences between the groups of patients were evaluated using nonparametric Mann-Whitney U test. Correlations between data were assessed with a Spearman rank test. Differences were considered statistically significant at $p < 0.05$.

Results

The demographics and clinical characteristics of the asthma and COPD patients are listed in table 1. Patients with COPD were significantly older than the group of asthmatics. Respiratory function tests showed a significantly stronger impairment of respiratory function in the COPD than in the asthma patients. The most significant differences were found in the pack-years ($p = 0.0001$) and FEV₁/FVC ($p = 0.0017$).

A significantly higher IL-6 level was found in the induced sputum of COPD patients than in the asthma subjects ($p = 0.00001$) (table 2; fig. 1). There were no significant differences in the level of IL-13 between the asthma and COPD groups. Medians had the same values in the

Table 1. Characteristics of patients

	Asthma patients (n = 18)	COPD patients (n = 26)	Statistical analysis asthma/COPD, p value
Male	3	22	
Female	15	4	
Age, years	45.8 ± 13.21	67 ± 8.2(54–81)	0.000005
Atopy, n	15	0	
Pack-years	5.6 ± 10.9 (0–40)	31.6 ± 19 (0–60)	0.0001
FEV ₁ , l	2.52 ± 1.03 (1.42–5.12)	1.67 ± 0.71 (0.68–3.22)	0.0044
FEV ₁ % predicted	84.89 ± 15.84 (61–110)	65.73 ± 19.65 (25–108)	0.0020
FVC, l	3.59 ± 0.94 (2.56–5.64)	2.92 ± 1.05 (1.46–4.89)	0.0338
FVC% predicted	103.4 ± 13.52 (76–123)	90.36 ± 18.23 (64–124)	0.0065
FEV ₁ /FVC	68.6 ± 10.93 (53–91)	56.56 ± 10.39 (31–67)	0.0017

Patient data are mean ± SD, values in parentheses are ranges.

Table 2. IL-6 and IL-13 levels in the induced sputum of asthmatic and COPD patients

Cytokine		Asthma patients (n = 18)	COPD patients (n = 26)	Statistical analysis asthma/COPD, p
IL-6, pg/ml	mean ± SD	21.5 ± 25.5	186.6 ± 145.4	0.000001
	range of values	0 – 105	18 – 473	
	median	13	149	
IL-13, pg/ml	mean ± SD	92.4 ± 110.5	75.8 ± 75.9	n.s.
	range of values	0 – 435	0 – 333	
	median	62.5	62.5	
IL-6/IL-13	mean ± SD	0.524 ± 0.644	3.392 ± 3.791	0.000025
	range of values	0.009 – 2.1	0.22 – 14.25	
	median	0.26	1.895	

two patient groups, although the mean value was higher in the asthmatics.

IL-13 was undetectable in the induced sputum of 6 out of 18 and 6 out of 26 cases of asthma and COPD, respectively. IL-6 was detected in only 1 asthma patient.

Because of the potential role of IL-6 and presumed importance of IL-13 in obstructive respiratory disease, we compared the IL-6 to IL-13 ratio among the individuals of the two patient groups (fig. 2). In all cases of an undetectable level of an interleukin its concentration was arbitrarily set as 0.76 pg/l for IL-6 and 32 pg/ml for IL-13 (the respective detection limits of the ELISA kits). The mean values of the IL-6 to IL-13 ratio were 0.524 in asthma and 3.392 in COPD patients ($p = 0.000025$), and this difference was statistically significant regardless of whether the patients with undetectable levels of cytokines were included or omitted.

We also examined the potential association of respiratory function with IL-6 and IL-13 levels in induced sputum. The statistically significant correlations and those not reaching statistical significance but worth noticing are shown in figure 3. There were significant correlations between IL-6 and FEV₁ ($r = -0.59$, $p = 0.01$) and between IL-6 and FEV₁/FVC ($r = -0.54$, $p = 0.02$) in asthma patients. The correlations between IL-6 and FEV₁ or FVC in this group of patients were also negative ($r = -0.40$, $r = -0.29$, respectively) but did not reach statistical significance. Negative correlations, albeit not statistically significant were found in the COPD group between IL-6 and FEV₁, FEV₁% and FVC ($r = -0.30$, -0.30 and -0.38 ; $p = 0.15$, 0.15 and $p = 0.07$, respectively). Except for only 1 case of negative correlation between IL-13 level and FVC in the COPD group (statistically nonsignificant), there was no relationship between concentrations of IL-13 and impaired respiratory function.

Fig. 1. IL-6 and IL-13 levels in induced sputum from asthmatic and COPD patients. Horizontal lines indicate the mean value for each group.

Fig. 2. IL-6 to IL-13 ratio in the induced sputum of asthma and COPD patients. Range of values and medians are indicated. Please note the logarithmic scale.

Discussion

We found that the level of IL-6 was significantly higher in the induced sputum of COPD patients and it was associated with significantly lower values of respiratory tests in this patient group compared to asthmatic subjects. Our results are consistent with previously published studies which demonstrated that COPD exacerbation increased serum or sputum IL-6 [21, 22]. It was also previously reported that the serum concentration of IL-6 was associated [11] or correlated [23] with a reduction of FEV₁ in COPD patients. The studies undertaken to investigate the sputum cell and cytokine characteristics of COPD demonstrated higher levels of IL-6 in patients categorized as frequent exacerbation (≥ 3 exacerbation in the previous year) as compared to infrequent exacerbation (≤ 2 exacerbation in the previous year). In addition, the difference in IL-6 concentration between exacerbation and the stable stage of the disease was statistically significant [24]. An association between IL-6 in induced sputum and the severity of COPD has been confirmed elsewhere through a significant inverse correlation between the IL-6 value and FEV₁, FEV₁/FVC and disease duration. Generally, our findings support a negative correlation of respiratory function and IL-6 level in the induced sputum of COPD patients, although in our study the correlation coefficient did not reach a statistical significance.

IL-6 has been attributed to the proinflammatory activities in COPD as well as asthma [25]. The inflammation in allergic asthma is mainly due to the immunological memory of previous contact with the allergen. Generally, the expression of immunological memory leads to inflammation directed by Th2 cytokines promoting the production of other inflammatory mediators. Some data showed that in mild-moderate asthma IL-6 dissociates from other proinflammatory biomarkers and Neveu et al. [26] suggest that it could be related to impaired lung function. However, the association of IL-6 with respiratory function in asthma is not yet clearly established and only a few studies have addressed this issue. IL-6 is one of the inflammatory cytokines that is produced by lung epithelial cells after allergen stimulation [27]. Sputum IL-6 was inversely associated with lower levels of postbronchodilator FEV₁ in asthma and asthma with rhinitis [28]. Quite recently a strong negative correlation has been reported between FEV₁ and sputum IL-6 levels [29]. Our results clearly confirmed the relation of IL-6 level with respiratory function in asthma – the level of IL-6 was negatively correlated with FEV₁ and FEV₁/FVC.

The importance of the presence of IL-13 in the airways of patients with impaired respiratory function remains controversial [30]. Increased IL-13 is well documented in the specimens from the respiratory tract of asthmatics, but the data for COPD are conflicting [31].

Fig. 3. Correlations between IL-6 and IL-13 levels in the induced sputum and lung function of asthma and COPD patients. Statistical analysis was performed using Spearman's rank test.

Some studies indicate an association between IL-13 and an increased percentage of eosinophils. IL-13 can stimulate the overexpression of eotaxins in bronchial epithelium [32] and indirectly intensify the recruitment of eosinophils into bronchial space. An elevated level of eo-

taxin was detected in association with increased eosinophils and ECP in the sputum of asthmatic subjects compared with healthy subjects, which led the authors to conclude that eotaxin was involved in the pathogenesis of eosinophilic airway inflammation [33]. Numerous au-

thors have confirmed a stimulating effect of IL-13 on the production of eotaxins, which are the most effective eosinophil chemoattractants [34–37]. In addition, the number of eotaxin-positive cells was significantly higher in exacerbated chronic bronchitis and asthma when compared to healthy subjects [38]. Unfortunately, a possible association between IL-13 concentration and eosinophil number could not be analyzed in our study because the differentiation of sputum cells was only performed in a few patients.

In a cross-sectional cohort study Lee et al. [30] have shown inverse associations between plasma concentrations of IL-13 and the severity of symptoms in COPD patients, as measured by percentage of FEV₁ or DLCO. Our findings do not support these results and to some extent confirm the opposite results recently published by Saha et al. [6]. Those authors did not find a difference in induced sputum IL-13 concentration (only measurable in 6 out of 34 subjects) between COPD GOLD 2–4 and healthy controls [5]. In our study the sputum IL-13 was measurable in 20 out of 26 subjects with COPD and its concentration did not correlate with respiratory function except for an unexpected positive correlation with FEV₁/FVC.

In our study the level of IL-13 did not differ statistically between COPD and asthma although the mean value of IL-13 concentration was somewhat higher in asthma patients. We suppose this could be due to the low number of patients tested or, more likely, to the relatively low detection limit of IL-13 at –32 pg/ml, making the dif-

ferences between the COPD and asthma subjects undetectable below this value. It also cannot be excluded that the low levels of cytokines in asthma patients observed in our study were related to inhaled corticosteroids since the decrease of proinflammatory cytokine levels in sputum after inhaled corticosteroid therapy has been documented [39–41].

This is the first analysis of levels of IL-6 and IL-13 in the induced sputum of COPD and asthma using the same ELISA kits. Previously the cytokines in the two patient groups were evaluated separately and reported in relation to their levels in healthy subjects. Given an unclear relation of IL-13 with the pathophysiology of an obstructive pulmonary disease, it is worth noting a significant difference in the IL-6 to IL-13 ratio in induced sputum of asthma and COPD patients. The high predominance of IL-6 in COPD and the same balance between both cytokines and the predominance of IL-13 in asthma suggests a different immunological status between both groups patients. A definitive explanation of the role of IL-13 in the pathophysiology of obstructive pulmonary diseases requires further clinical trials.

In conclusion, our results strongly suggest that a high level of IL-6 but not IL-13 is associated with respiratory disorders in asthma patients. In the context of all the studies cited above and our results, IL-13 seems to be related to the pathogenicity due to the inflow of eosinophils into the airway observed in both asthma and COPD patients.

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