



Expression of Inflammatory Mediators in Induced Sputum: Comparative Study in Asthma and COPD

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Abstract

Asthma and COPD are the most common obstructive lung diseases characterized by inflammation in the lower airways which contribute to airflow limitation. Different inflammatory mediators are thought to play a key role in these diseases. This study was conducted in 13 patients with asthma, 12 patients with COPD, and 13 control subjects. The expression of mRNA of IL-6, IL-13, CXCL8, TSLP, IL-33, IL-25, IL-17, ECP, mast cell tryptase, CCL24, and CCL26 was assessed in induced sputum cells by real time PCR. We found that CXCL8 was strongly related to the neutrophil percentage but differed significantly in COPD and asthma patients. The expression of IL-17 was lower in patients with atopic asthma compared to non-atopic asthma. The percentage of macrophages correlated negatively with the expression of mast cell tryptase and ECP in

COPD, and with CXCL8 in asthma. The expression of ECP correlated negatively with the severity of COPD symptoms measured by CAT. We conclude that asthma and COPD demonstrate a significant overlap in the airway cytokine profile. Thus, differentiation between the two diseases is difficult as based on a single cytokine, which suggests the coexistence of phenotypes sharing a common cytokine network in these obstructive lung diseases.

Keywords

Airway • Asthma • COPD • Eosinophils • CXCL8 • IL-17 • IL-6 • Inflammation • Mediators • Neutrophils • Respiration

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1 Introduction

Asthma and COPD are complex airway diseases that share some common features including clinical presentation and pulmonary function impairment. Although both diseases are associated with chronic airway inflammation, they differ in terms of the inflammatory cells and mediators involved. The immunopathology of asthma is characterized by peribronchial infiltration of eosinophils, macrophages, and

T lymphocytes. Eosinophils are thought the major inflammatory cells in asthma but the role of mast cells and T-helper cells is also well established (Bradding 2008; Moqbel and Odemuyiwa 2008). The influx of neutrophils into the airways observed in severe asthma may be a predictor of steroid resistance (Macedo et al. 2009). Neutrophils also are the most abundant inflammatory cells present in the bronchial wall and lumen of COPD patients. Increased airway neutrophilia correlates with lung function decline, and characterizes acute exacerbations of COPD (Singh et al. 2010; Fujimoto et al. 2005).

As the spectrum of clinical presentations and other features of asthma and COPD is wide, different phenotypes have been defined in order to better characterize both diseases, their mechanisms, and the effect of different therapies. The type of airway inflammation defined by the predominant inflammatory cells is one of the most important factors defining asthma phenotypes. Even though the major COPD phenotypes have been defined by clinical and radiological presentations, features of airway inflammation and remodeling may also be used to cluster the disease (Górska et al. 2010; Han et al. 2010).

Identification of biological markers reflecting the underlying disease process also seems to be important. Parallel to the differences in the cellular composition of the inflammatory infiltrates in asthma and COPD, different mediators are thought to play the main role in both diseases. In patients with COPD, markers of systemic inflammation can be measured in peripheral blood but these markers do not seem to sufficiently reflect the inflammatory processes within the airways (Röpcke et al. 2012). Thus, a reliable assessment of local airway inflammation in asthma and COPD must be based on biological samples taken from the site of ongoing inflammation. Recently evaluated mediators of asthma include interleukin 25 (IL-25), IL-33, thymic stromal lymphopoietin (TSLP), and periostin (Bobolea et al. 2015; Byers 2014), while neutrophil-associated proteins, including neutrophil elastase and matrix metalloproteinases in sputum have been reported as potential markers of airway remodeling in COPD (Paone et al. 2011; Vernooij et al. 2004). However, there are as yet no

established and recommended biomarkers that would reliably reflect the type and severity of airway inflammation (Koutsokera et al. 2013).

Induced sputum (IS) has been extensively studied as a non-invasive sampling method useful in the evaluation of the cellular pattern and the concentration of different inflammatory mediators. However, protein levels of various inflammatory mediators measured by commercially available kits are often undetectable in IS supernatant, being below test sensitivity. The use of dithiothreitol for IS processing can further decrease the concentration of many proteins. The assessment of mRNA expression may be a more sensitive method for mediator evaluation in IS (Gelder et al. 1995). Some data show that mRNA expression may better reflect airway hyperresponsiveness and airflow limitation than sputum eosinophilia (Jung et al. 2014). IL-4, IL-5, and IL-13 mRNA expression in IS cells can be used to classify asthma into Th₂-high and Th₂-low endotypes (Peters et al. 2014).

Based on the data presented above we undertook a study aimed to: 1/compare the mRNA expression of different inflammatory proteins IL-6, IL-13, chemokine (C-X-C motif) ligand 8 (CXCL8), thymic stromal lymphopoietin, IL-33, IL-25, IL-17, eosinophil cationic protein (ECP), mast cell tryptase, eotaxin-2 (CCL24), eotaxin-3 (CCL26) in cells obtained from IS of asthma and COPD patients and 2/characterize the relationship between the expression of mRNA of different mediators and sputum cellular phenotypes of asthma and COPD.

2 Methods

2.1 Characteristics of Patients

The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/275/2012) and informed written consent was obtained from all the participants. The study involved 13 patients with asthma, 12 patients with COPD, and 13 control subjects. All asthma and COPD patients were stable and free from exacerbations for at least 8 weeks

before the study onset. None of patients or control subjects have respiratory infections for at least one month preceding the enrollment.

Asthma was diagnosed according to the GINA recommendations (GINA 2015). Atopic status was assessed by skin prick testing with a panel of fifteen aeroallergens. Asthma control test (ACT) was performed in all asthma patients. Short-acting β 2-agonists (SABA) on demand was the only treatment in eight patients, one patient was treated only with long acting β 2-agonists (LABA). Two patients received low doses of inhaled corticosteroids (ICS) and LABA, while two others received moderate doses of ICS and LABA.

COPD was diagnosed according to the GOLD recommendations (GOLD 2015). COPD assessment test (CAT) and modified Medical Research Council (mMRC) Questionnaire were used to measure severity of symptoms. Two COPD patients were treated with a low dose of ICS plus LABA, five patients with LABA and long-acting muscarinic antagonists (LAMA), four patients received LABA and short-acting muscarinic antagonists (SAMA) one used LABA only.

The control group consisted of healthy non-smoking subjects, with normal results of spirometry.

2.2 Sputum Induction

Sputum induction was preceded by premedication with 400 μ g of inhaled salbutamol. After postbronchodilator spirometry the patients inhaled sterile hypertonic saline (NaCl) at increasing concentrations (3 %, 4 %, and 5 % solutions, 10 min per inhalation) *via* an ultrasonic nebulizer (ULTRA-NEB TM 2000, DeVilbiss, USA). After each inhalation, spirometry was performed to detect a potential forced expiratory volume in 1 s (FEV₁) decrease. The procedure was stopped when a significant (≥ 20 %) FEV₁ decline as compared to postbronchodilator baseline value was noted (Chmielowicz et al. 2008).

Induced sputum was processed immediately on receipt as described by other authors (Hargreave et al. 1998). The volume of sputum was measured and the plugs were separated and weighed. A freshly prepared 0.1 % solution of dithiothreitol (Sigma Aldrich; St. Louis, USA) was added in a

volume equal to four the weight of the sputum, and the mixture was shaken for 15 min. Subsequently, 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, sputum was centrifuged for 10 min at 1800 *g*. The cells were counted, and the percentage of dead and epithelial cells was assessed. The pellet of cells was suspended in RNAlater solution (Qiagen; Valencia, USA) and stored at -80 °C for further investigation. The smears were stained with MG-Giemsa staining. The criteria for appropriate IS quality were as follows: a minimum of 2 ml of expectoration; less than 50 % squamous epithelial cells, and more than 300 non-epithelial cells on one slide.

2.3 RNA Isolation and cDNA Synthesis

Total RNA was isolated using Nucleo Spin RNA II Columns Kit (Machery & Nagel; Düren Germany). The concentration and purity of isolated RNA was measured on a DU650 spectrophotometer (Beckman Coulter, Brea, USA). Eight microliters of total RNA was used for reverse transcription using SuperScript III First-Strand Synthesis Super Mix for qRT-PCR (Invitrogen, Carlsbad, USA).

2.4 Real-Time Quantitative PCR

For real-time PCR, 0.8 μ l of cDNA was amplified in 16 μ l PCR volume, containing a Power SYBR Green PCR mastermix (Applied Biosystems, Foster City, CA) with 150 nM of specific primers. Sequences of the applied primers are shown in Table 1. PCR protocol consisted of one cycle at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Each sample was measured in duplicate. 18S rRNA was applied for each sample as an internal control in order to normalize gene expression levels. The results were expressed as relative quantification (RQ) units (fold change). The PCR evaluation was performed with an ABI-Prism 7500 Sequence Detector System (Applied Biosystems, Foster City, CA).

Table 1 Sequence of primers used in PCR

	Forward primer	Reverse primer	Product size
18 s rRNA	GGATGAGGTGGAACGTGTGAT	AGGTCTTCACGGAGCTTGTG	148
IL-6	CCGGGAACGAAAGAGAAGCT	GCGCTTGTGGAGAAGGAGTT	67
IL-13	TCAACATCACCCAGAACCAGAA	AGCTGTCAGGTTGATGCTCCATA	70
IL-8	GAGCACTCCATAAAGGCACAAACT	ATCAGGAAGGCTGCCAAGAG	149
ECP	TTTGCCATCCAGCACATCA	TTAATTGCCCGCATTGCA	61
Mast cell tryptase	CACCGCCATTTCTCTGAAG	GCGTCACAAATGTGGTTTTCC	60
IL-17	AGGAATCACAATCCCACGAAAT	GGTGAGGTGGATCGGTTGTAGT	149
IL-25	TGGTCCCTTTTTGGGAAACC	TGTGCAGAAGTGCAGGCTTT	153
IL-33	GCCTAGATGAGACACCGAATTAACA	CCAGGGTCAGAAGGGATGGT	85
TSLP	TCTTGAATTTCCGCTGCAA	CCACTGGTGTATAGGGTTCTGA	78
CCL24	CACATCATCCCTACGGGCTCT	GTTGCCAGGATATCTCTGGACAGGG	88
CCL26	GGAAGTCCACACGTGGGAGTGAC	CTCTGGGAGGAAACACCCTCTCC	354

IL interleukin, ECP eosinophil cationic protein, CCL24 eotaxin-2, CCL26 eotaxin-3, TSLP thymic stromal lymphopoietin

Relative quantification values were calculated by the $2^{-\Delta\Delta CT}$ method. The cycle threshold (CT) for the target amplicon and CT for endogenous control (18S rRNA) were determined for each sample. Differences were calculated between these two CTs and called ΔCT , in order to account for the difference in the amount of total nucleic acid added to each reaction. The values of ΔCT for cDNA from sputum cells of healthy subjects (calibrator) were subtracted from the ΔCT of each sample and termed $\Delta\Delta CT$. The target normalized to endogenous control ratio, relative to the calibrator, was then calculated by the formula $2^{\Delta\Delta CT}$. The calculation was made, CT of the cDNA of sputum cells from patients with asthma or COPD was calibrated with the average CT of sputum cells from healthy volunteers.

2.5 Statistical Analysis

Results are given as medians and range of values. Differences between continuous variables were tested using the nonparametric Mann-Whitney U or Kruskal-Wallis test. Correlations between variables were analyzed with Spearman's rank test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using Statistica 9.0 software (StatSoft Inc., Tulsa, OK).

3 Results

Baseline patients' characteristics are presented in Table 2. In the asthma group, 8, 3, and 2 patients had mild, moderate, and severe disease, respectively. The degree of disease control was as follows: well-controlled asthma – 7 patients, partly controlled asthma – 4 patients, and uncontrolled asthma – 2 patients. The median value of ACT was 20 (range 11–25) points.

The number of COPD patients classified as GOLD A, GOLD B, and GOLD D category was 4, 6, and 2 respectively. The use of spirometric classification of COPD severity based on post-bronchodilator FEV₁ value revealed 11 patients with moderate (GOLD 2) and one patient with very severe (GOLD 4) disease (Rabe et al. 2007). The median value of CAT was 15.5 (range 4–23) points.

Patients with COPD were significantly older and had a significantly greater tobacco smoke exposure than healthy volunteers and asthma patients. Both asthma and COPD patients had impaired lung function (lower FEV₁/FVC and FEV₁% predicted) as compared to controls. Significantly greater impairment of respiratory function (lower FEV₁% predicted) was demonstrated in COPD than in asthma patients.

The cellular composition of IS in asthmatics, controls, and COPD patients is shown in Table 3. The percentage of lymphocytes and epithelial

Table 2 Demographic and clinical data of patients with asthma, COPD, and healthy controls

	Asthma	COPD	Controls	p
Gender (male/female)	1/12	7/5	7/6	NA
Atopy (n)	8	3	0	NA
Age (yr)	53 (22–75)	70 (59–82)	42 (31–64)	0.0030 ^{#◇}
Smoking (pack-years)	0 (0–10)	55 (14–100)	0 (0–20)	0.0001 ^{#◇}
FEV ₁ (L/s)	2.9 (1.1–3.1)	1.5 (0.5–1.8)	3.9 (2.0–5.1)	0.0001*
FEV ₁ %pred.	88 (45–121)	51 (21–78)	106 (89–125)	0.0001 [#]
FVC (L)	4.1 (2.4–4.5)	2.7 (1.5–3.8)	4.6 (2.8–7.0)	0.0001 [◇]
FVC %pred.	105 (83–139)	93 (45–124)	113 (84–132)	0.0082 [◇]
FEV ₁ /FVC (%)	69 (46–84)	45 (36–61)	81 (70–96)	0.0001 ^{#◇}

COPD chronic obstructive pulmonary disease, FEV₁ forced expiratory volume in one second, FVC forced vital capacity, NA non-applicable

Data are medians (range); p < 0.05, significant differences for paired comparisons (Kruskal-Wallis test): [#]asthma vs. COPD, *asthma vs. control, [◇]COPD vs. control

Table 3 Percentage of epithelial and inflammatory cells in induced sputum (IS) of asthmatics, COPD patients, and controls

	Asthma	COPD	Controls	p
Epithelial cells	22 (8–48)	14 (4–37)	13 (7–45)	0.45
Macrophages	27.5 (14–65)	12 (0–34)	33 (7–61)	0.07 [◇]
Lymphocytes	5.5 (1–13)	8 (1–16)	6 (1–15)	0.22
Neutrophils	52.5 (17–78)	74 (54–91)	55 (30–90)	0.03 ^{#◇}
Eosinophils	3.5 (1–28)	3 (0–10)	1 (0–1)	0.001 ^{#◇}

The sum of all non-epithelial cells was taken as 100 %

Data are presented as medians (range); p < 0.05, significant differences for paired comparisons (Kruskal-Wallis test): *asthma vs. control, [#]asthma vs. COPD, [◇]COPD vs. control

cells was similar in all groups. However, the percentage of macrophages and neutrophils was significantly higher in COPD patients than those in controls and asthmatics. The highest and lowest percentages of eosinophils were found in the asthma patients and controls, respectively.

Of all the measured gene expressions only CXCL8 differed significantly between COPD and asthma patients: 3.9 (range 0.2–54.5) vs. 0.8 (range 0.2–162.2) (RQ units), respectively (Fig. 1). The analysis of asthma and COPD patients subclassified according to the IS eosinophil and neutrophil percentage showed a significantly elevated CXCL8 expression in asthmatics with neutrophilia ($\geq 60\%$) as compared with the remaining asthma patients [1.3 (range 0.8–7.9) vs. 0.4 (range 0.2–162.2) (RQ units), p < 0.045]. We observed increased FEV₁/FVC [74 (range 68–84) vs. 67 (range 46–88) (%), p < 0.045] in neutrophilic as compared to non-neutrophilic asthmatics (Table 4). No other significant differences in pulmonary function parameters in the asthma and COPD groups classified according the eosinophil/

neutrophil phenotype of sputum were found. CXCL8 expression in IS was insignificantly lower in COPD patients with high percentage of eosinophils ($\geq 3\%$) than in COPD patients with a lower eosinophil percentage ($< 3\%$) [2.2 (range 0.2–13.5) vs. 9.4 (range 3.0–54.5) (RQ units), p = 0.07].

The analyses within asthma and COPD groups showed some relationships between mRNA expression and patients' characteristics. In the non-atopic asthmatics, IL-17 expression was significantly higher [1.4 (range 0.6–13.0) (RQ units)] than in the atopic ones [0.5 (range 0.2–1.4) (RQ units), p = 0.05] (Fig. 2). In COPD, CAT score correlated significantly with ECP expression ($r = -0.69$, p = 0.01) (Fig. 3). None of the evaluated mediators correlated with age either in asthma or COPD.

The percentage of macrophages correlated negatively with the expression of almost all evaluated mediators, except for IL-33 and CCL26 in asthma, IL-6 and IL-13 in COPD, and CCL24 in both diseases. This correlation

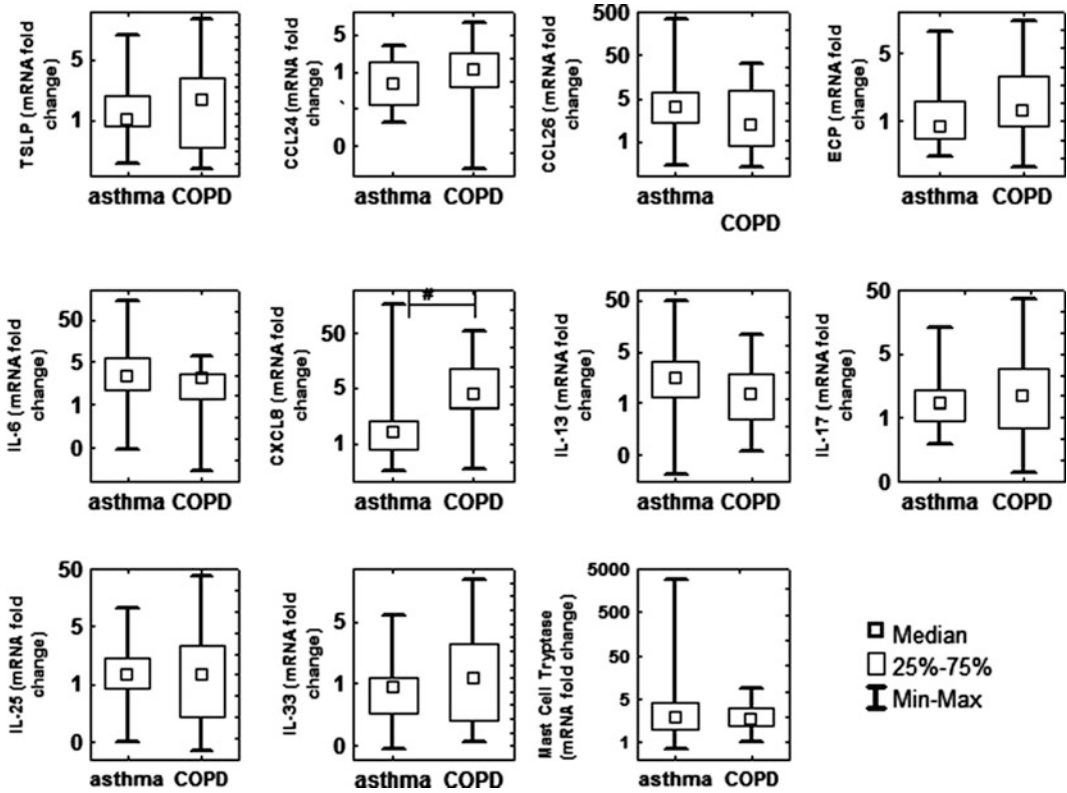


Fig. 1 Box plots of expression of CCL24, CCL26, ECP, IL-13, IL-25, IL-33, TSLP, mast cell tryptase, IL-17, IL-6, and CXCL8 in induced sputum of asthma and COPD patients (logarithmic scale). # $p < 0.05$ (Mann-Whitney U test)

Table 4 Expression of chemokine (C-X-C motif) ligand 8 (CXCL8) in induced sputum (IS) and spirometry in asthma and COPD patients stratified according to eosinophil or neutrophil predominance

Asthma	Non-eosinophilia vs. eosinophilia			Non-neutrophilia vs. neutrophilia		
	<3 % eosinophils n = 6	≥3 % eosinophils n = 7	p	<60 % neutrophils n = 5	≥60 % neutrophils n = 8	p
CXCL8 (RQ)	1.0 (0.4–4.2)	0.4 (0.2–162.2)	0.14	0.4 (0.2–162.2)	1.3 (0.8–7.9)	0.045*
FEV ₁ % pred.	82 (45–121)	83 (65–93)	0.95	77 (45–93)	86 (69–121)	0.280
FEV ₁ /FVC (%)	71 (46–84)	69 (52–75)	0.53	67 (46–75)	74 (68–84)	0.045*
COPD	Non-eosinophilia vs. eosinophilia			Non-neutrophilia vs. neutrophilia		
	<3 % eosinophils n = 7	≥3 % eosinophils n = 5	p	<60 % neutrophils n = 3	≥60 % neutrophils n = 9	p
CXCL8 (RQ)	9.4 (3.0–54.5)	2.2 (0.2–13.5)	0.07	2.2 (0.9–13.5)	4.7 (0.2–54.5)	0.73
FEV ₁ % pred.	47 (21–78)	54 (48–74)	0.80	65 (58–74)	50 (21–78)	0.06
FEV ₁ /FVC (%)	42 (36–6)	50 (41–55)	0.58	51 (50–55)	44 (36–61)	0.21

Data are medians (range); COPD chronic obstructive pulmonary disease, RQ relative quantification units, FEV₁ forced expiratory volume in one second, FVC forced vital capacity; * $p < 0.05$, significant differences between neutrophilia vs. non-neutrophilia in asthmatics (Mann-Whitney U test)

Fig. 2 Interleukin (IL)-17 expression in induced sputum of asthma patients with and without atopy (Mann-Whitney *U* test)

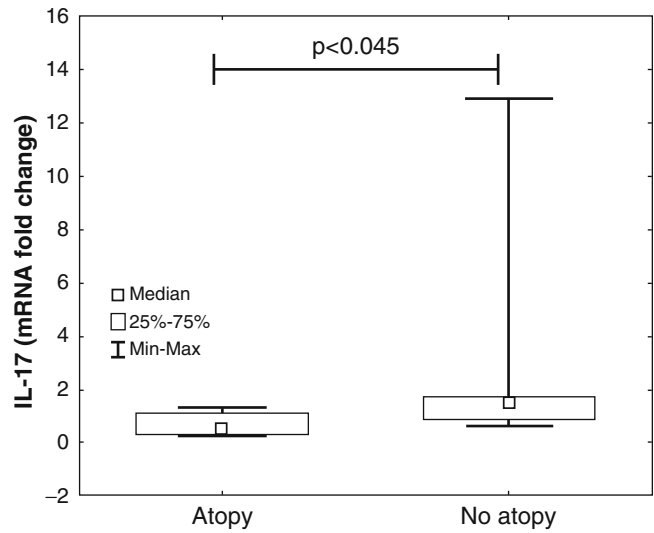
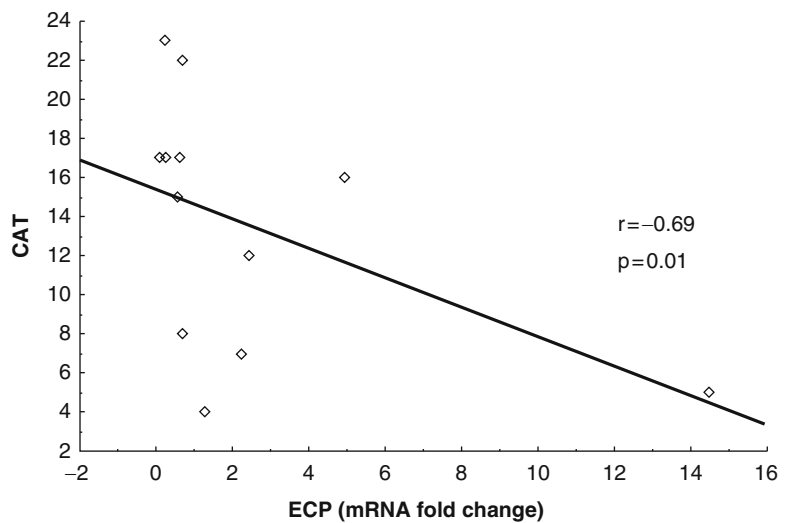


Fig. 3 Correlations between eosinophil cationic protein (ECP) expression in induced sputum and disease assessment test (CAT) score in COPD patients (Spearman’s rank correlation)



was significant in three cases only: mast cell tryptase in COPD ($r = -0.70, p = 0.01$), CXCL8 in asthma ($r = -0.72, p < 0.01$), and ECP in COPD ($r = -0.60, p = 0.04$) (Table 5).

4 Discussion

This study evaluated the expression of eleven different mediators in induced sputum cells from asthma and COPD patients. We found that CXCL8 was the only cytokine with a

significantly different expression in asthma and COPD. There were no appreciable differences in terms of cytokine expression in IS cells in patients with different asthma or COPD phenotypes based on clinical characteristics. The exception was IL-17 whose level was significantly lower in atopic asthmatics compared with non-atopic ones. We also found that the neutrophilic phenotype was characterized by a higher CXCL8 mRNA level compared with the eosinophilic phenotype, irrespective of the underlying disease. An interesting observation was a

Table 5 Correlations between expressions of CXCL8, IL-6, IL-8, IL-33, IL-25, TSLP, IL-17, mast cell tryptase, IL-13, ECP, CCL24, and CCL26 and macrophage percentage in induced sputum (IS) of asthma and COPD patients

Macrophages (%)	Asthma	COPD
CXCL8	$r = -0.72, p = 0.01$	$r = -0.06, p = 0.86$
IL-6	$r = -0.17, p = 0.55$	$r = 0.41, p = 0.19$
IL-33	$r = 0.13, p = 0.96$	$r = -0.30, p = 0.34$
IL-25	$r = -0.36, p = 0.25$	$r = -0.45, p = 0.14$
TSLP	$r = -0.35, p = 0.26$	$r = -0.48, p = 0.11$
IL-17	$r = -0.31, p = 0.28$	$r = -0.40, p = 0.19$
Mast cell tryptase	$r = -0.27, p = 0.35$	$r = -0.70, p = 0.01$
IL-13	$r = -0.13, p = 0.66$	$r = 0.04, p = 0.90$
ECP	$r = -0.49, p = 0.09$	$r = -0.60, p = 0.04$
CCL24	$r = 0.08, p = 0.80$	$r = 0.54, p = 0.07$
CCL26	$r = 0.01, p = 0.96$	$r = -0.05, p = 0.87$

COPD chronic obstructive pulmonary disease, *CXCL8* chemokine (C-X-C motif) ligand 8, *IL* interleukin, *TSLP* thymic stromal lymphopoietin, *ECP* eosinophil cationic protein, *CCL24* eotaxin-2, *CCL26* eotaxin-3. Statistical analysis was performed using Spearman's rank test

negative correlation between ECP expression in IS and the severity of COPD symptoms measured by CAT.

Elevated CXCL8 expression in COPD is consistent with the results of earlier studies which have reported higher CXCL8 concentrations in sputum and bronchoalveolar lavage fluid of COPD patients compared with asthmatics (Hollander et al. 2007; Yamamoto et al. 1997). CXCL8, a strong neutrophil chemoattractant, is produced by macrophages, dendritic cells, bronchial epithelial cells and airway smooth muscle cells particularly after bacterial or pro-inflammatory stimulation (e.g., TNF- α , LPS, or cigarette smoke) (Paplińska et al. 2011; Sarir et al. 2010; Gosens et al. 2009). In the present study, CXCL8 expression in IS positively correlated with neutrophil and inversely with eosinophil percentage. There is evidence that sputum neutrophilia is associated with more severe asthma phenotype and disease exacerbations (Moore et al. 2014; Paone et al. 2011), and with impaired lung function in COPD and asthma (Shaw et al. 2007; Boulet et al. 2003). We failed to find significant differences between the degree of airflow limitation in patients with eosinophilic vs. non-eosinophilic and neutrophilic vs. non-neutrophilic phenotypes of asthma or COPD. The only exception was a higher FEV₁/FVC in asthmatics with IS neutrophilia compared with asthma patients

without IS neutrophilia. However, albeit FEV₁/FVC was slightly higher in asthmatics with IS neutrophil percentage $\geq 60\%$, there were no significant differences in terms of FEV₁% of predicted value. We believe that the lack of significant differences between pulmonary function parameters in patients with neutrophilic vs. non-neutrophilic disease in the present study might be, in part, explained by a rather mild nature of asthma in 8 (62%) out of the 13 patients; there were only 2 (15%) patients with severe asthma. In earlier studies, the relationship between IS neutrophils and impaired lung function was reported mainly in patients with severe asthma. For instance, Little et al. (2002) have observed that sputum neutrophil count and activation was negatively associated with the maximal FEV₁ value obtained after treatment with oral steroids or maximal dose of fluticasone in combination with salbutamol.

Asthma and COPD are heterogeneous diseases with various inflammatory phenotypes. That may explain the lack of significant differences in the expression of the majority of the cytokines investigated. Although we carefully selected patients for this study and enrolled only patients with unequivocal clinical signs and symptoms of asthma or COPD, the cellular composition of IS in these two groups was variable, with some COPD patients presenting an increased percentage of eosinophils. The role of

these cells in COPD is a matter of discussion. Some studies have shown that COPD might be associated with eosinophil predominance in the airways (10–40 % of COPD subjects), which is particularly pronounced during COPD exacerbations (Siva et al. 2007). Induced sputum eosinophilia in COPD has also been reported in our previous work (Gorska et al. 2008). The results of the present study suggest that the degree of eosinophilic inflammation in stable COPD, measured by ECP expression in IS, may inversely correlate with the severity of symptoms assessed by CAT. That is consistent with our previous study that has indicated that expression of the eosinophil chemoattractant CCL26 in nasal mucosa could be a negative regulator for neutrophil airway infiltration and was strongly positively correlated with FEV₁/FVC in COPD patients (Paplińska et al. 2012). The assumption is that a higher eosinophil percentage would be related to a lower number of neutrophils in IS and, in consequence, to the lack of a negative influence of neutrophil airway infiltration on lung function. This assumption is strengthened by the observation that neutrophils rather than eosinophils are associated with lung function decline and irreversible airway obstruction (Boulet et al. 2003).

The only significant relation between the expression of pro-inflammatory cytokines in IS and clinical phenotypes of asthma and COPD was low IL-17 expression in patients with atopic asthma. As the role of Th17 cells, main source of IL-17, in atopy is not fully understood, the significance of this finding is unclear. IL-17 promotes neutrophilic influx into the airways in the allergic mode of asthma (Sergejeva et al. 2005). At the same time, IL-17 strongly promotes IgE production (Milovanovic et al. 2010). Some authors have shown a decreased number of circulating Th17 cells in patients with atopic dermatitis (Hayashida et al. 2011), while others have shown elevated IL-17 expression in IS from patients with allergic asthma (Manise et al. 2013). The present results suggest that Th2 and Th17 pathways can directly antagonize each other and that atopy status is associated with lower IL-17 expression in IS in asthma.

The present study suggests that macrophage function is dysregulated in obstructive lung diseases. Given the fact that macrophages are important effector cells which influence the course of asthma and COPD, we assume that dysfunction of these cells could play a role in the pathogenesis of obstructive lung diseases. Although our study demonstrates a weak negative correlation between the percentage of macrophages in IS and the expression of mediators, except for IL-13 in asthma, CCL26 in COPD, and CCL24 in both, we believe these results should be highlighted. According to the literature, macrophage function is impaired in asthma and COPD. Hodge et al. (2003) have shown that alveolar macrophages from COPD patients have a reduced ability to phagocytose apoptotic bronchial epithelial cells. It has also been reported that macrophage phagocytosis is reduced in non-eosinophilic asthma (Simpson et al. 2013). In yet another study, eosinophilic inflammation in asthma has been associated with decreased CD16 expression in bronchial macrophages (Moniuszko et al. 2007). The relationship between airway hyperresponsiveness and dysfunction of bronchial macrophages has been confirmed in mild asthma (Alexis et al. 2001). The plausibility arises that neutrophilia in severe asthma and COPD might be a consequence of reduced and inefficient phagocytotic and efferocytotic function (clearance of apoptotic cells) of macrophages. The prolonged state of ineffective airway clearance enhances bacterial colonization (Matkovic and Miravittles 2013). In the present study, expression of ECP in COPD patients correlated negatively with the total percentage of macrophages in IS. ECP is produced by monocytes, not macrophages (Byström et al. 2001) and is thought to attract macrophages (Liu et al. 2012b). We suppose that this correlation could be associated with M1 phenotype of macrophages, which plays a role in the response to bacterial colonization, often present in the airways of patients with COPD (Patel et al. 2002).

The present investigation has several limitations. Firstly, the study groups were relatively small. Secondly, some patients with

asthma and COPD were treated with low or moderate doses of ICS, which may affect both the cellular composition of IS and the expression of different cytokines mRNA. Inhaled ICS reduce eosinophil count (van Rensen et al. 1999), airway hyperresponsiveness (Liu et al. 2012a), improve lung function (Jatakanon et al. 1998) and decrease the level of inflammatory mediators in asthmatic airways (Basyigit et al. 2004). On the other hand, ICS have no or meager anti-inflammatory action in stable COPD and neutrophilic asthma (Green et al. 2002; Culpitt et al. 1999). As our intention was to evaluate patients with stable disease (relatively well balanced pro-inflammatory and anti-inflammatory activity), treatment with ICS was not the exclusion criterion. In fact, patients treated with ICS are usually not excluded from studies that evaluate various aspects of airway inflammation (Peters et al. 2014; Sanchez-Cuellar et al. 2012). We indeed observed significantly lower ECP and IL-25 expression in the whole group of patients compared with those without ICS treatment, but a small number of ICS-treated patients made a further interpretation of this observation not feasible. Thirdly, the same control group was used for comparisons with asthma and COPD. As there were some differences between patients with asthma and COPD (e.g., age), the control group could not be well-matched to the patients of both diseases. As a result, there were significant differences between some features of COPD and control subjects (e.g., age or tobacco exposure). We accepted this drawback because the major aim of our study was to compare the data from asthma and COPD patients. We believe that a comparison of results from COPD patients with those from non-smoking control subjects, representing normal airway environment, might provide data of interest.

5 Conclusions

The present study demonstrates CXCL8 as the only cytokine in induced sputum showing significantly different expression between asthma

and COPD. There is a significant overlap in expression of cytokines in patients with asthma and COPD. CXCL8 expression was related to the percentage of neutrophils in both COPD and asthma, which makes differentiation between the two diseases hard on the basis of a single cytokine expression. The corollary is that many common phenotypes share similar cytokine network in these obstructive lung diseases. Interestingly, higher expression of eosinophil mediators in induced sputum correlated with better COPD control as assessed by CAT. Macrophage phenotypes and macrophage-derived cytokines involved in the pathogenesis of asthma and COPD as well as the influence of eosinophils on COPD severity should be further explored in detail using alternative study designs.

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