Respiratory Medicine (2007) **101**, 1947–1953

ELSEVIER

respiratoryMEDICINE

Serum and bronchial lavage fluid concentrations of IL-8, SLPI, sCD14 and sICAM-1 in patients with COPD and asthma

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Received 16 November 2006; accepted 13 April 2007
Available online 15 June 2007

KEYWORDS
Asthma;
COPD;
Inflammatory biomarkers;
Bronchial lavage fluid;
Serum

Summary
Background: Airway inflammation is associated with an increased expression and release of inflammatory reactants that regulate processes of cell migration, activation and degranulation. The purpose of this study was to quantify bronchial lavage (BAL) fluid and serum levels of chemokine (IL-8), secretory leukocyte protease inhibitor (SLPI), soluble intracellular adhesion molecules-1 (sICAM-1) and sCD14, as surrogate markers of inflammatory and immune response in asthma and chronic obstructive pulmonary disease (COPD) patients with similar disease duration time.
Methods: Biomarkers in serum and BAL fluid from asthma ($n = 13$) and COPD ($n = 25$) patients were measured using commercially available ELISA kits.
Results: We found that in asthma and COPD groups the concentrations of IL-8 and SLPI are significantly higher in BAL fluid than in serum, while levels of sICAM-1 and sCD14 in BAL fluid are significantly lower than in serum. Of these 4 measured biomarkers, only the BAL IL-8 was higher in COPD patients when compared to asthma ($P<0.05$). In both groups, BAL IL-8 correlated with SLPI ($r = 0.577$, $P<0.01$ and $r = 0.589$, $P<0.05$, respectively). In patients with COPD the BAL sICAM-1 correlated with sCD14 ($r = 0.576$, $P<0.01$), while in asthma patients BAL sICAM-1 correlated with FEV_1/FVC ($r = 0.418$, $P<0.01$). Moreover, in asthma patients the serum SLPI correlated with sCD14 ($r = 0.688$, $P<0.01$) and serum sICAM-1 negatively correlated with FEV_1/FVC ($r = -0.582$, $P<0.05$).
Conclusion: Our findings point to the importance of selecting a correct biological fluid when analyzing specific biomarkers, and also show that of 4 measured biomarkers, only the BAL IL-8 was higher in COPD patients when compared to asthma.

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0954-6111/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.rmed.2007.04.010

Introduction

Airway inflammation is a cardinal pathophysiological feature in patients with asthma and chronic obstructive pulmonary disease (COPD). Both diseases are associated with an increased expression and release of inflammatory reactants, including cytokines, chemokines, proteases and their inhibitors, and adhesion molecules that regulate processes of cell migration, activation and degranulation.[1,2] Recent studies show similar cellular responses in asthma and COPD, with raised levels of neutrophils in more severe forms of asthma[3,4] and raised counts and activation of eosinophils in COPD.[5–7] Monocytes/macrophages are a significant component of the inflammatory infiltrate in COPD[8] and several studies show that monocytes/macrophages are in an activated state in asthma and orchestrate immune reactions.[9,10] For instance, levels of CD14, a marker of monocyte/macrophage activation,[11] have been shown to be increased in asthma patients during acute asthma attacks.[12–14]

IL-8 occurs at high levels in COPD and is a selective attractant of neutrophils and IL-8 levels in induced sputum are correlated with the extent of neutrophilic inflammation and with disease severity (% predicted FEV_1).[15,16] IL-8 has also been suggested to play a role in asthma, where it is reported to be involved in lymphocyte, eosinophil and basophil activation and migration to the inflammatory site.

Adhesion molecules are important in the recruitment and migration of leukocytes from circulation to the inflammatory sites. Elevated cell surface expression of intercellular adhesion molecule-1 (ICAM-1) has been demonstrated in asthmatic airways[17,18] and increased levels of soluble ICAM-1 (sICAM-1) has been found in asthmatic sputum.[19] Whether ICAM-1 is up-regulated to the same extent in COPD remains uncertain, as the studies published so far have yielded conflicting results.[18–20]

Proteases and their inhibitors are also implicated in the pathophysiology and airway pathology of COPD as well as asthma.[21] Secretory leukocyte protease inhibitor (SLPI), also known as antileukoprotease, is a naturally occurring serine proteinase inhibitor produced by mucosal epithelial cells, serous cells, and bronchiolar goblet cells in human airways.[22,23] Historically, this inhibitor was first purified from lung secretions of patients with COPD and cystic fibrosis. However, emerging evidence suggests that SLPI is also important in the pathophysiology of asthma because it exhibits broad-spectrum inhibitory activity against mast cell and leukocyte serine proteases implicated in asthma pathology.[24–26]

Thus, inflammation in the respiratory tract involves epithelial cells, resident macrophages, eosinophils, and neutrophils, and provides many cell-released markers that can be used to follow pathophysiological changes related to the disease. The majority of protein expression changes are closely associated with many aspects of the pathophysiology of disease, including inflammation, airway remodelling, tissue damage and repair, mucus production, and plasma infiltration.[27] However, an understanding of the relationship between the complex array of cells and mediators involved in asthma and COPD is not yet fully dissected which makes difficult to find a specific and sensitive panel of biomarkers that can reflect intensity of these pathological processes and can help to predict the individual outcome. It is well known that the validation of specific biomarkers requires the choice of appropriate biological medium for analysis, therefore we aimed to measure and to compare serum and BAL fluid concentrations of SLPI, sICAM-1, IL-8 and sCD14 -as potential inflammatory markers in asthma and COPD patients.

Materials and methods

Subjects

The studied group consisted of COPD ($n = 25$) and asthma ($n = 13$) hospital outpatients, age >35 years from the Department of Pulmonology and Immunology, Kaunas Medical University Hospital, Lithuania. The COPD diagnosis was based on the classification of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.[28] Patients included in the study had a forced expiratory volume in one second (FEV_1) of $<80\%$ of predicted and a FEV_1/FVC (forced vital capacity) ratio of $<70\%$ of predicted, classified as moderate ($n = 13$) to severe ($n = 12$) COPD (GOLD stages II–III). In to the study there were included COPD patients with a smoking history (current or ex-smoker) of $\geqslant 10$ pack-years or never-smokers ($n = 4$) who have exposure to occupational dust and chemicals.

Adult asthmatics with a history of asthma of 6 months or more were diagnosed according to the standards of the Global Initiative for Asthma.[29] All patients were steroid-naïve or without inhaled and systemic steroids for at least 1 month before the study. None of the subjects showed signs of acute respiratory infection at least 1 month before the investigation.

The exclusion criteria were α1-antitrypsin deficiency, dyspnea of other origin (including cardiovascular disorders, pneumonia, interstitial lung disease, pleural disease, upper airways obstruction, neuromuscular disease and anaemia) and bronchial carcinoma. Number of smoking pack-years was calculated as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (year's $\times$ cig. per day/20). The study was approved at the Regional Bioethics Committee (protocol no. 48/2004) and written informed consent was received from all participants.

Lung function testing

Pulmonary function was tested using a pneumotachometric spirometer "Custo vitM" (Custo Med, Germany) with subjects in the sitting position, and the highest value of FEV_1 and FVC from at least two technically satisfactory maneuvers differing by less than 5% was recorded. Normal values were characterized according to Quanjer.[30] Subjects had to avoid the use of long-acting β_2-agonists for at least 48 h and short-acting β_2-agonists for at least 8 h prior to the test.

Bronchoscopy and bronchoalveolar lavage processing

Bronchoscopy was performed after the blood sampling and lung function testing. Subjects were not allowed to drink or eat at least 4 h prior the bronchoscopy. Smoking was not allowed at least 10 h before the procedure. To perform BAL, the local upper airways were anaesthetized with 5 ml of 2% Lidocaine (Grindex, Latvia). All bronchoscopic examinations were performed in the morning. The bronchoscope (Olympus, USA) was wedged into the segmental bronchus of the middle lobe and 5 boluses of 20 ml sterile saline solution at 37 °C was infused. Fluid was gently aspirated immediately after the infusion had been completed and was collected into a sterile container with a negative pressure of −40 to 50 mmHg. The fluid was immediately filtered using 48 μm nylon gauze (Millipore, North Ryde, NSW, Australia) and centrifuged at −4 °C for 10 min. Supernatants were removed and frozen in Eppendorf tubes at −70 °C for further investigation. All patients underwent bronchoscopic procedures without any significant complication.

Serum samples

Blood samples were drawn in serum tubes, clotted at room temperature for 30–60 min and centrifuged for 10 min at 3900 rpm. Serum samples were immediately frozen at −70 °C for further assay.

Analysis of serum and bronchial lavage (BAL) fluid

The serum and BAL fluid concentrations of IL-8, sICAM-1, sCD14 and SLPI were determined using commercially available quantitative enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Minneapolis, USA) according to the manufacturer's instructions. Assays were performed in duplicate and the optical density at 450 nm, with a background correction at 570 nm, was determined using a microplate reader (Labsystems iEMS Reader MF). The readings for each standard and sample were averaged and the average of the zero standard was subtracted. The detection limits of the IL-8, sICAM-1, sCD14-1 and SLPI kits were 31.2, 15.6, 125 and 62.5 pg/ml, respectively.

Analysis of total protein in BAL fluid

The protein concentration in the lavage fluids was measured by Bradford protein assay according to manufacturer's recommendations (Sigma, USA).[31]

Statistical analysis

Statistical analysis was performed using the SPSS software (version 12.0.1 for Windows, SPSS Inc., Chicago, IL, USA). Normal distribution of the variables was tested using the Kolmogorov–Smirnov test and since log-transformation of non-normally distributed variables did not improve the normal distribution, non-parametric tests were used. The Kruskal–Wallis test was used for comparisons between more than two groups and if significance was reached, the Mann–Whitney *U* test, with correction for multiple comparisons (Bonferroni), was used for group-wise comparisons. Correlations were investigated using the Spearman rank order correlation test. Data is presented as mean and range.

Results

Patient characteristics

Table 1 gives the demographic data, disease duration, lung function and smoking habits of the investigated asthma and COPD patients. The asthma patients were younger than the COPD group ($P = 0.026$), had more females than males (69.2% vs. 8.7%, respectively) and included significantly fewer smokers. Out of 25 COPD patients, 4 were never smokers and 11 ex-smokers. As expected, COPD patients had lower FEV_1 (FEV_1% of predicted 58.3 vs. 84.1, $P<0.001$) compared to asthma patients. No significant difference was found between asthma and COPD patients in disease duration time (8.6±2.5 vs. 7.8±1.3 years), serum AAT levels (1.47±5.6 vs. 1.38±5.4 mg/ml) and the concentration of total BAL fluid proteins.

Concentrations of IL-8, SLPI, sICAM-1 and sCD14 in BAL and serum

The concentration of BAL IL-8 was significantly higher in the group with COPD than in the group with asthma (mean±SE: 0.104±0.03 vs. 0.03±0.01 ng/ml, $P = 0.04$). No significant difference was observed between the two groups in serum IL-8 concentrations, although the mean levels were higher in COPD than in the asthma patients (mean±SE: 0.09±0.07 vs. 0.016±0.01 ng/ml). In both groups IL-8 concentrations were significantly higher in BAL fluid than in serum (COPD $P = 0.011$ and asthma $P = 0.034$).

As shown in Table 2, we found no significant difference in BAL and serum SLPI, sICAM-1 and sCD14 concentrations

Table 1 Patient demographics.

Diagnose	Asthma	COPD
Number of patients	13	25
Females/males	9/4	2/23
Age (years)	54.3±11	64±8.8
Duration of the disease (years)	8.6±2.5	7.8±1.3
Smoking history		
Never smokers	12	4
Smokers	1	10
Ex-smokers	0	11
BAL fluid recovery (ml)*	79.85±2.3	65.92±2.11
BAL total protein (ng/ml)	62.9±11.9	52.4±6.7
FEV_1% of predicted†	84.1±25	58.3±10
FVC% of predicted	91±20	75±17
FEV_1/FVC% ratio	77.4±7.7	60±9

All other data is presented as means±SD.
*Data is presented as means±SE.
†Measured after bronchodilatation.

Table 2 Concentrations of measured variables in serum and BAL fluid.

Measured protein (ng/ml)	COPD ($n = 25$)		Asthma ($n = 13$)	
	Serum	BAL	Serum	BAL
SLPI	40.34 (26.1–65)*	72.58 (0.4–250)	41.95 (28.6–60.1)	64.7 (17.9–116.8)
IL-8	0.09 (0.001–1.78)	0.104[a] (0.002–0.56)	0.016 (0.001–0.13)	0.03[a] (0–0.11)
sICAM-1	153 (92–224)	4 (0–15.3)	161 (102.4–314)	6.8 (0.9–14.4)
sCD14	1531 (750–2180)	363 (0–2667)	1433 (1065–1859)	292 (0.5–3091)

*Values are expressed as mean and (range).
[a]Welsh t-test shows significant difference in BAL IL-8 concentration between COPD and asthma patients $t(28.3) = 2.1$, $P = 0.04$.

Table 3 Serum and BAL fluid variables correlation matrix.

Variable	Group	Serum IL-8	Serum SLPI	Serum ICAM-1	Serum sCD14	BAL IL-8	BAL SLPI	BAL ICAM-1	BAL sCD14	FEV_1/FVC
Serum	Asthma	–	–	–	–	–	–	–	–	–
IL-8	COPD		–	–	–	–	–	–	–	–
Serum	Asthma	–			0.688**	–	–	–	–	–
SLPI	COPD	–			–	–	–	–	−0.429*	0.408*
Serum	Asthma	–	–	–	–	–	–	–	–	0.582*
ICAM-1	COPD	–	–	–	–	–	–	–	–	–
Serum	Asthma	–	0.688**	–	–	–	–	–	–	–
sCD14	COPD	–	–	–	–	–	–	–	–	–
BAL	Asthma	–	–	–	–	–	0.589*	–	–	–
IL-8	COPD	–	–	–	–	–	0.577**	–	–	–
BAL	Asthma	–	–	–	–	0.589*	–	–	–	–
SLPI	COPD	–	–	–	–	0.577**				
BAL	Asthma	–	–	–	–	–	–	–	–	0.418**
ICAM-1	COPD	–	–	–	–		–	–	0.576**	
BAL	Asthma	–	–					–		
sCD14	COPD	–	−0.429*	–	–	–	–	0.576**	–	–
	Asthma	–	–	0.582*	–	–	–	0.418**	–	–
FEV_1/FVC	COPD	–	0.408*	–	–	–	–		–	–

**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.
–Non-significant.

between the studied groups. However, SLPI concentration was higher in BAL fluid compared to serum in both COPD (72.6 vs. 40.3, statistically non-significant) and asthma (64.7 vs. 41.95, $P = 0.04$) groups. In contrast, in both groups levels of sICAM-1 and sCD14 in serum were higher compared to BAL (COPD: 152.8 vs. 4 pg/ml, $P<0.001$ and asthma: 161 vs. 6.8, $P<0.01$) and (COPD: 1531 vs. 363.2 pg/ml, $P<0.001$ and asthma: 1433 vs. 292, $P<0.01$), respectively.

Relationship between studied biomarkers in the group with asthma and COPD

As shown in Table 3, in patients with asthma the serum concentrations of SLPI significantly correlated with serum sCD14 whereas the BAL concentration of SLPI correlated with BAL IL-8 ($r = 0.589$, $P<0.05$). Interestingly, FEV_1/FVC showed a negative correlation with serum concentration of sICAM-1 ($r = -0.582$, $P<0.05$), but a positive correlation with BAL fluid sICAM-1 ($r = 0.418$, $P<0.05$). In the group with COPD, the BAL concentrations of IL-8 significantly correlated with BAL SLPI and BAL concentrations of sICAM-1 correlated with BAL sCD14 (Table 3). In addition, negative correlation was found between BAL concentration of sCD14 and serum SLPI ($r = -0.429$, $P<0.05$) and positive correlation between serum SLPI concentration and FEV_1/FVC ($r = 0.408$, $P<0.05$). No association was observed between measured biomarkers and smoking habit.

Discussion

Previously it has been demonstrated that IL-8 levels are increased in COPD patients,[15,32] and that increased levels of IL-8 in sputum samples correlate with the airway bacterial load and proteinase released from activated neutrophils.[33]

Recently, Schulz and collaborators[34] have shown that in subjects with COPD the constitutive and stimulated IL-8 release is significantly higher compared to 'healthy' smokers and control subjects. Consistent with this, we also found higher concentrations of BAL fluid IL-8 in the patients with COPD than in those with asthma. It is important to note that our patient groups differ significantly in age, gender, and smoking habits; i.e. the asthma group includes predominantly never-smoking females while the COPD group is primarily smoking males. Thus, it is possible that the higher IL-8 levels in COPD patients are related to the above differences between the patient groups. It has been demonstrated that cigarette smoke induces the release of IL-8 from cultured human bronchial epithelial cells,[35] and BAL specimens from non-asthmatic smokers have greater concentrations of neutrophils, macrophages, and a number of cytokines, including IL-1β, IL-6, IL-8, and monocyte chemoattractant protein-1 than non-smokers.[36] So far, the differences and similarities in the pathological processes of COPD patients with and without a smoking history are not known. It is important to point out that 4 out of 25 COPD patients included in our study were never smokers and 11 ex-smokers. However, we found no association between BAL fluid or serum IL-8 levels and smoking history in the COPD group, suggesting that the mechanism for IL-8 release may depend on several factors, and not just simply on smoking alone. Furthermore, the BAL IL-8 level did not correlate with age and gender. The molecular mechanisms that trigger, maintain, and regulate the expression and release of IL-8 have not yet been fully elucidated. For instance, bacterial endotoxin and tumor necrosis factor-alpha (TNF-α) are known to increase IL-8 production by epithelial cells.[37] It has been proposed that neutrophil elastase may also stimulate epithelial cells to produce more IL-8.[38] Neutrophil elastase has also been shown to increase SLPI transcription in primary human airway epithelial cells,[24,26] and we found a direct correlation between BAL fluid IL-8 and SLPI concentrations in both COPD and asthma groups. However, SLPI, like IL-8, may also be up-regulated by bacterial endotoxins and cytokines such as IL-1 and TNFi.[39,40]

Inflammation in COPD and asthma extends beyond the airways,[41,42] therefore the biomarkers reflecting systemic rather than local inflammation in the lungs may be of greater value in diagnosis. In support of this, the results of Little and collaborators[43] show that high levels of plasma intercellular adhesion molecule-1 (sICAM-1, CD54) and IL-8 in BAL fluid correlate with the development and severity of chronic lung disease. Increased levels of sICAM-1 in plasma and BAL from patients with COPD have been reported.[44–46] Serum concentrations of sICAM-1 were also found to be increased in acute asthma.[47] We found no significant difference in serum and BAL fluid sICAM-1 concentrations between the COPD and asthma groups. However, it is important to note that in patients with asthma the FEV_1/FVC ratio showed direct correlation with BAL sICAM-1 but inverse correlation with serum sICAM-1. This finding is difficult to interpret, but it has been shown that in untreated asthmatics, ICAM-1 is over-expressed in bronchial epithelial cells.[18] The asthma patients included in our study were untreated for at least 1 month prior to sample collection and were in stable condition. This may explain why sICAM-1 was found to be correlated to lung function in asthma patients. In the COPD group, BAL fluid sICAM-1 correlated with soluble sCD14, an acute phase protein, mainly released from activated monocytes/macrophages and hepatocytes.[48] CD14-dependant mechanisms of inflammation have been discussed in acute respiratory distress syndrome,[49] sarcoidosis[50] and asthma.[51] The soluble form of CD14 is generated by proteolytic shedding of the membrane-associated form (mCD14) during cellular activation and can be detected proportionately in the BAL fluid.[52] We found significantly higher levels of sCD14 as well as sICAM-1 in serum compared to BAL.

In general, many markers of inflammation in BAL fluid may have a stronger signal than the same markers in blood and vice versa, therefore the information gained from such assays might be difficult to interpret. For instance, in the present study we have found that in asthma and COPD groups the concentrations of IL-8 and SLPI are significantly higher in BAL fluid than in serum while levels of sICAM-1 and sCD14 in BAL fluid are significantly lower than in serum. It is well known that the major sites of SLPI synthesis are Clara and goblet cells of the surface epithelium,[53,54] and the serous cells of the sub mucosal glands.[55–57] Therefore, it is reasonable to believe that analysis of serum SLPI may only partially reflect the ongoing pathological process in the airways, and therefore too insensitive to monitor the actual state of disease. The relevance of circulating IL-8, sICAM-1 and sCD14 and their relation to locally production in COPD and asthma remain much less clear. Thus, the applicability of these biomarkers in respiratory diseases will much depend on the selection of a correct biological fluid when analyzing. Another important point, beside the compartment, is the biological reasoning to measure the specific biomarker. In order to avoid spurious association, the selected biomarker must fit to the current knowledge of the biochemical basis of the disorder.

Author's contributions

CH carried out the analysis, participated in the interpretation of data and helped to draft the manuscript. BS and RS collected patient material. UW helped in the interpretation of data and in the design of the study. SJ designed the study, carried out the final statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work has been supported by grants from the Thelma Zoega's and Crafoords Foundations, the Swedish Heart Lung Foundation, the Kaunas Medical University Science Foundation.

References

1. O'Byrne PM, Postma DS. The many faces of airway inflammation. Asthma and chronic obstructive pulmonary disease. Asthma Research Group. *Am J Respir Crit Care Med* 1999;**159**(5 Part 2):S41–63.
2. Barnes PJ. Mechanisms in COPD: differences from asthma. *Chest* 2000;**117**(2 Suppl):10S–4S.

3. Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma: persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 1997;**156**(3 Part 1):737–43.
4. Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med* 1999;**160**(5 Part 1):1532–9.
5. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997;**155**(2):449–53.
6. Balzano G, Stefanelli F, Iorio C, De Felice A, Melillo EM, Martucci M, et al. Eosinophilic inflammation in stable chronic obstructive pulmonary disease: relationship with neutrophils and airway function. *Am J Respir Crit Care Med* 1999;**160**(5 Part 1): 1486–92.
7. Gibson PG, Fujimura M, Niimi A. Eosinophilic bronchitis: clinical manifestations and implications for treatment. *Thorax* 2002;**57**(2):178–82.
8. Tetley TD. Macrophages and the pathogenesis of COPD. *Chest* 2002(121):156S–9S.
9. Tomita K, Tanigawa T, Yajima H, Fukutani K, Matsumoto Y, Tanaka Y, et al. Identification and characterization of monocyte subpopulations from patients with bronchial asthma. *J Allergy Clin Immunol* 1995;**96**(2):230–8.
10. Demoly P, Simony-Lafontaine J, Chanez P, Pujol JL, Lequeux N, Michel FB, et al. Cell proliferation in the bronchial mucosa of asthmatics and chronic bronchitics. *Am J Respir Crit Care Med* 1994;**150**(1):214–7.
11. Kusunoki T, Nakahata T, Miyanomae T, Inoue Y. Possible dual effect of CD14 molecule on atopy. *Am J Respir Crit Care Med* 2002;**165**(4):551–2 [author reply, p. 552].
12. Virchow Jr JC, Julius P, Matthys H, Kroegel C, Luttmann W. CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma. *Eur Respir J* 1998;**11**(2):317–23.
13. Lapa e Silva JR, Possebon da Silva MD, Lefort J, Vargaftig BB. Endotoxins, asthma, and allergic immune responses. *Toxicology* 2000;**152**(1–3):31–5.
14. Garty BZ, Monselise Y, Nitzan M. Soluble CD14 in children with status asthmaticus. *Isr Med Assoc J* 2000;**2**(2):104–7.
15. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;**153**(2): 530–4.
16. Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokuyama T, Tsukaguchi K, et al. Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest* 1997;**112**(2):505–10.
17. Louis R, Bettiol J, Cataldo D, Sele J, Henquet M, Radermecker M. Effect of a 4-week treatment with theophylline on sputum eosinophilia and sputum eosinophil chemotactic activity in steroid-naive asthmatics. *Clin Exp Allergy* 2000;**30**(8):1151–60.
18. Vignola AM, Campbell AM, Chanez P, Bousquet J, Paul-Lacoste P, Michel FB, et al. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. *Am Rev Respir Dis* 1993;**148**(3):689–94.
19. Louis R, Shute J, Biagi S, Stanciu L, Marrelli F, Tenor H, et al. Cell infiltration, ICAM-1 expression, and eosinophil chemotactic activity in asthmatic sputum. *Am J Respir Crit Care Med* 1997;**155**(2):466–72.
20. Di Stefano A, Maestrelli P, Roggeri A, Turato G, Calabro S, Potena A, et al. Upregulation of adhesion molecules in the bronchial mucosa of subjects with chronic obstructive bronchitis. *Am J Respir Crit Care Med* 1994;**149**(3 Part 1):803–10.
21. Sallenave JM. The role of secretory leukocyte proteinase inhibitor and elafin (elastase-specific inhibitor/skin-derived antileukoprotease) as alarm antiproteinases in inflammatory lung disease. *Respir Res* 2000;**1**(2):87–92.
22. Thompson RC, Ohlsson K. Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. *Proc Natl Acad Sci USA* 1986;**83**(18):6692–6.
23. Eisenberg SP, Hale KK, Heimdal P, Thompson RC. Location of the protease-inhibitory region of secretory leukocyte protease inhibitor. *J Biol Chem* 1990;**265**(14):7976–81.
24. Abbinante-Nissen JM, Simpson LG, Leikauf GD. Neutrophil elastase increases secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. *Am J Physiol* 1993;**265**(3 Part 1):L286–92.
25. Maruyama M, Hay JG, Yoshimura K, Chu CS, Crystal RG. Modulation of secretory leukoprotease inhibitor gene expression in human bronchial epithelial cells by phorbol ester. *J Clin Invest* 1994;**94**(1):368–75.
26. Sallenave JM, Shulmann J, Crossley J, Jordana M, Gauldie J. Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. *Am J Respir Cell Mol Biol* 1994;**11**(6):733–41.
27. Wu J, Kobayashi M, Sousa EA, Liu W, Cai J, Goldman SJ, et al. Differential proteomic analysis of bronchoalveolar lavage fluid in asthmatics following segmental antigen challenge. *Mol Cell Proteomics* 2005;**4**(9):1251–64.
28. Global Initiative for Chronic Obstructive Lung Disease. *Global strategies for the diagnosis, management and prevention of chronic obstructive lung disease*, 2004, 〈http://www.goldcopd.com〉.
29. *GINA: global initiative for asthma*, 〈http://www.ginasthma.com〉.
30. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and coal. Official statement of the European Respiratory Society. *Eur Respir J Suppl* 1993;**16**: 5–40.
31. Stoscheck CM. Quantitation of protein. *Methods Enzymol* 1990;**182**:50–68.
32. Nocker RE, Schoonbrood DF, van de Graaf EA, Hack CE, Lutter R, Jansen HM, et al. Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 1996;**109**(2):183–91.
33. Mikami M, Llewellyn-Jones CG, Stockley RA. The effect of interleukin-8 and granulocyte macrophage colony stimulating factor on the response of neutrophils to formyl methionyl leucyl phenylalanine. *Biochim Biophys Acta* 1998;**1407**(2):146–54.
34. Schulz C, Wolf K, Harth M, Kratzel K, Kunz-Schughart L, Pfeifer M. Expression and release of interleukin-8 by human bronchial epithelial cells from patients with chronic obstructive pulmonary disease, smokers, and never-smokers. *Respiration* 2003; **70**(3):254–61.
35. Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *Am J Respir Crit Care Med* 1997;**155**(5):1770–6.
36. Kuschner WG, D'Alessandro A, Wong H, Blanc PD. Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur Respir J* 1996;**9**(10):1989–94.
37. Khair OA, Devalia JL, Abdelaziz MM, Sapsford RJ, Tarraf H, Davies RJ. Effect of *Haemophilus influenzae* endotoxin on the synthesis of IL-6, IL-8, TNF-alpha and expression of ICAM-1 in cultured human bronchial epithelial cells. *Eur Respir J* 1994;**7**(12):2109–16.
38. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest* 1992; **89**(5):1478–84.

39. Martinez FO, Sironi M, Vecchi A, Colotta F, Mantovani A, Locati M. IL-8 induces a specific transcriptional profile in human neutrophils: synergism with LPS for IL-1 production. *Eur J Immunol* 2004;**34**(8):2286–92.
40. Suarez EC, Lewis JG, Krishnan RR, Young KH. Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. *Psychoneuroendocrinology* 2004;**29**(9):1119–28.
41. Sevenoaks MJ, Stockley RA. Chronic obstructive pulmonary disease, inflammation and co-morbidity—a common inflammatory phenotype? *Respir Res* 2006;**7**:70.
42. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax* 2004;**59**(7):574–80.
43. Little S, Dean T, Bevin S, Hall M, Ashton M, Church M, et al. Role of elevated plasma soluble ICAM-1 and bronchial lavage fluid IL-8 levels as markers of chronic lung disease in premature infants. *Thorax* 1995;**50**(10):1073–9.
44. Rutgers SR, Postma DS, ten Hacken NH, Kauffman HF, van Der Mark TW, Koeter GH, et al. Ongoing airway inflammation in patients with COPD who do not currently smoke. *Thorax* 2000;**55**(1):12–8.
45. Marguet C, Dean TP, Warner JO. Soluble intercellular adhesion molecule-1 (sICAM-1) and interferon-gamma in bronchoalveolar lavage fluid from children with airway diseases. *Am J Respir Crit Care Med* 2000;**162**(3 Part 1):1016–22.
46. Riise GC, Larsson S, Lofdahl CG, Andersson BA. Circulating cell adhesion molecules in bronchial lavage and serum in COPD patients with chronic bronchitis. *Eur Respir J* 1994;**7**(9): 1673–7.
47. Tang RB, Chen SJ, Soong WJ, Chung RL. Circulating adhesion molecules in sera of asthmatic children. *Pediatr Pulmonol* 2002;**33**(4):249–54.
48. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990;**249**(4975):1431–3.
49. Martin TR, Rubenfeld GD, Ruzinski JT, Goodman RB, Steinberg KP, Leturcq DJ, et al. Relationship between soluble CD14, lipopolysaccharide binding protein, and the alveolar inflammatory response in patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1997;**155**(3):937–44.
50. Striz I, Zheng L, Wang YM, Pokorna H, Bauer PC, Costabel U. Soluble CD14 is increased in bronchoalveolar lavage of active sarcoidosis and correlates with alveolar macrophage membrane-bound CD14. *Am J Respir Crit Care Med* 1995;**151**(2 Part 1):544–7.
51. Dubin W, Martin TR, Swoveland P, Leturcq DJ, Moriarty AM, Tobias PS, et al. Asthma and endotoxin: lipopolysaccharide-binding protein and soluble CD14 in bronchoalveolar compartment. *Am J Physiol* 1996;**270**(5 Part 1):L736–44.
52. Kleeberger SR, Peden D. Gene–environment interactions in asthma and other respiratory diseases. *Annu Rev Med* 2005;**56**:383–400.
53. Kramps JA, Willems LN, de Water R, Franken C, Dijkman H. Bronchial secreting cells and antileukoprotease. In: Junod A, Oliveri D, Pozzi E, editors. *Endothelial and mucus secreting cells*. Milan: Masson; 1991. p. 235–45.
54. Willems LN, Kramps JA, Stijnen T, Sterk PJ, Weening JJ, Dijkman JH. Antileukoprotease-containing bronchiolar cells: relationship with morphologic disease of small airways and parenchyma. *Am Rev Respir Dis* 1989;**139**(5):1244–50.
55. Mooren HW, Meyer CJ, Kramps JA, Franken C, Dijkman JH. Ultrastructural localization of the low molecular weight protease inhibitor in human bronchial glands. *J Histochem Cytochem* 1982;**30**(11):1130–4.
56. Saitoh H, Masuda T, Shimura S, Fushimi T, Shirato K. Secretion and gene expression of secretory leukocyte protease inhibitor by human airway submucosal glands. *Am J Physiol Lung Cell Mol Physiol* 2001;**280**(1):L79–87.
57. De Water R, Willems LN, Van Muijen GN, Franken C, Fransen JA, Dijkman JH, et al. Ultrastructural localization of bronchial antileukoprotease in central and peripheral human airways by a gold-labeling technique using monoclonal antibodies. *Am Rev Respir Dis* 1986;**133**(5):882–90.