

The effect of induced sputum and bronchoalveolar lavage fluid from patients with chronic obstructive pulmonary disease on neutrophil migration in vitro

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Key words: neutrophils; migration; chronic obstructive pulmonary disease; induced sputum; bronchoalveolar lavage fluid.

Summary. *Objective.* The aim of study was to investigate a chemotactic effect of induced sputum and bronchoalveolar lavage fluid on blood neutrophils in patients with chronic obstructive pulmonary disease (COPD) and healthy individuals.

Material and methods. Forty-three smokers with COPD, 19 ex-smokers with COPD, 13 healthy smokers, and 17 healthy nonsmokers were recruited to the study.

Neutrophils were isolated from peripheral blood of study individuals. For the same experimental conditions, pooled induced sputum and bronchoalveolar lavage fluid of 20 COPD patients were used.

Neutrophil chemotaxis in vitro was performed in cell-transmigration chamber. Substances tested for chemoattraction (interleukin-8, induced sputum, bronchoalveolar lavage fluid directly or in addition to interleukin-8) were added to lower wells. Upper wells were filled with 2.5×10^6 /mL of neutrophil culture and incubated for 2 hours. Migration was analyzed by flow cytometry.

Results. Interleukin-8 (10–100 ng/mL) induced a dose-dependant neutrophil migration in all the groups. Only 100 ng/L of interleukin-8 induced more intensive chemotaxis of neutrophils from COPD smokers as compared to ex-smokers ($P < 0.05$). Such difference between healthy individuals was obtained using 30 ng/mL of interleukin-8 ($P < 0.05$).

Induced sputum/interleukin-8 (10–100 ng/mL), as well as induced sputum directly, induced neutrophil migration ($P < 0.05$). Chemotaxis of neutrophils isolated from COPD patients and healthy nonsmokers did not depend on additional interleukin-8 concentration.

Bronchoalveolar lavage fluid/interleukin-8 (30–100 ng/mL) induced more intensive migration of neutrophils from COPD patients than bronchoalveolar lavage fluid ($P < 0.05$) alone.

Conclusions. Migration of neutrophils isolated from patients with COPD was more intensive compared to healthy individuals. Induced sputum and bronchoalveolar lavage fluid directly and with addition of interleukin-8 stimulated chemotaxis, and it was higher in neutrophils from COPD patients. Migration of neutrophils did not depend on smoking status.

Introduction

Neutrophils are key cells in inflammatory response of chronic obstructive pulmonary disease (COPD), which is characterized by poorly reversible airflow limitation. It is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases (1). Accumulation of neutrophils in the airways is recognized as a prominent feature of COPD, with the extent of neutrophilic infiltration both in the airways and tissues correlating with disease severity (1–3).

Reactivity of neutrophils to chemoattractive signals, known as chemotaxis, is crucial for an ef-

ficient control of pathogens. It is a biological phenomenon whereby a cell migrates through barriers (vessel walls or epithelial layers) and tissues toward a site of inflammation or infection (4–5). Excessive transmigration of neutrophils from blood into the airway, lung tissues and abnormal activation are largely responsible for the overproduction of reactive oxygen species (ROS), proteases and inflammatory cytokines, which play an important role in lung injury (6–8). However, still little is known about the cellular and molecular mechanisms that control neutrophil migration to the airways during COPD.

The causes of COPD are multifactor, involv-

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ing genetic and environmental factors. Smoking is a major environmental risk factor for COPD (1, 9, 10). It is hypothesized that smoking directly activates the maturation of blood neutrophils and induces neutrophils and macrophages to release neutrophil chemotactic factors such as interleukin-8 (IL-8) (11). Levels of IL-8 have been shown to be higher in induced sputum of smokers compared to nonsmokers (1, 11, 12). While tobacco smoke primarily targets the lungs, COPD is a disease that exerts effects remote from the lungs. Thus, COPD is a systemic inflammatory disorder that influences the peripheral leukocytes and plasma proteins, leading to a cascade of systemic inflammatory events.

IL-8, the concentration of which especially increases in induced sputum and bronchoalveolar lavage (BAL) fluid during COPD (1, 6), is a major important chemokine and chemoattractant of neutrophils, produced and released by neutrophils (11, 13), alveolar macrophages (14), and other activated cells. IL-8 induces the release of myeloperoxidase from neutrophils and contributes to further recruitment of inflammatory cells, helping to sustain inflammation (15).

Despite well-known chemotactic features of IL-8, it is unknown if other components of induced sputum and BAL fluid may modulate the chemotaxis of peripheral blood neutrophils. As the number of neutrophils highly increases in induced sputum and BAL fluid during COPD (1–3, 11), other chemoattractants should be present in induced sputum and BAL fluid as well. However, a direct influence of these substances on chemotaxis of peripheral blood neutrophils is unknown.

Our previous study (16) has shown that induced sputum and BAL fluid from COPD patients are able to modulate ROS production of peripheral blood neutrophils. Thus, the aim of this study was to investigate a potentially chemotactic role of induced sputum and BAL fluid on neutrophil migration and its dependence on smoking status in COPD patients and healthy individuals.

Material and methods

Study individuals and design. A total of 62 patients with stable moderate-to-severe COPD

(according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease) and 30 healthy individuals were recruited to the study.

All individuals met the following criteria: had not used inhaled and systemic steroids at least 1 month before the study and had smoking history of more than 10 pack-years. None of the subjects showed signs of acute respiratory infection at least a month before the investigation.

All COPD patients were screened for deficiency of alpha-1 antitrypsin (AAT), and none of the patients had Z allele, which may cause genetically determined deficiency of AAT.

Study individuals were divided into subgroups according to smoking status (Table 1). COPD patients were divided into smokers and ex-smokers and healthy individuals to smokers and nonsmokers.

Subgroups were formed according to these criteria: 1) smokers, currently smoking persons, having more than 10 pack-years smoking history; 2) ex-smokers, persons having smoking history of more than 10 pack-years, who ceased smoking more than 2 years before the study; 3) nonsmokers, individuals who have never smoked.

The Kaunas Regional Ethics Committee for Biomedical Research approved the study, and written informed consent was obtained from all individuals.

Lung function testing. Pulmonary function was tested using a pneumotachometric spirometer “CustovitM” (Custo Med, Germany) with individuals in the sitting position, and the highest value of forced expiratory volume in 1 sec (FEV₁) and forced vital capacity (FVC) from at least two technically satisfactory maneuvers differing by less than 5% was recorded. Individuals had to avoid the use of short-acting β_2 -agonists at least 8 hours before the test.

Sample preparation. *Neutrophil isolation from peripheral blood.* Peripheral blood samples were collected into sterile vacutainers with ethylene diamine tetra-acetic acid (EDTA), and neutrophils were isolated by a density gradient centrifugation. The whole blood was layered on Ficoll-Paque PLUS (GE Healthcare, Finland) and centrifuged at 1000g for 30 min at room temperature. Neutrophil population was separated using a hypotonic erythrocyte lysis. Isolated neutrophils were diluted in cell culture

Table 1. General data of study individuals

Characteristic	COPD		Healthy individuals	
	Smokers	Ex-smokers	Smokers	Nonsmokers
Individuals, n	43	19	13	17
Sex, male/female	33/10	13/6	8/5	12/5
Mean age, years	58.6±7.1	57.3±6.6	53.8±9.0	50.0±7.4
Smoking pack-years	28±6	31±4	26±5	0

Data are presented as mean±SEM; n, number of individuals.

RPMI 1640 (Biological Industries, Israel) media to a final concentration of 2.5×10^6 /mL.

The viability of neutrophils was checked by flow cytometry, and it was always more than 95%.

Induced sputum and BAL fluid samples. Induced sputum and BAL fluid samples were prepared according to standardized guidelines (17). Briefly, sputum induction was performed using an ultrasonic nebulizer (DeVilbiss Health Care, USA) via inhalation of 3% sterile hypertonic saline solution (Ivex Pharmaceuticals, USA). Followed by 5-min inhalation, patients were encouraged to cough an adequate amount of sputum.

BAL fluid was obtained during bronchoscopy in a week after sputum induction procedure. The bronchoscope (Olympus, Japan) was wedged into the segmental bronchus of the middle lobe, and 140 mL of 0.9% sterile saline solution was infused. Fluid was gently aspirated immediately after the infusion and was collected into a sterile container.

Induced sputum and BAL fluid samples were processed immediately after recovery or sputum expectoration, according to standardized guidelines (17). Suspensions were centrifuged at 200g within 20 min, and supernatants were stored at -70°C .

In order to assure the same conditions for experimental chemotaxis analysis, pooled and filtered induced sputum and BAL fluid supernatants of 20 COPD patients were used.

Neutrophil transmigration assay. Neutrophil chemotaxis in vitro was performed in 10-well cell transmigration chamber (Neuro Probe, USA). The lower and upper wells of chamber were isolated by a polyvinylpyrrolidone (PVP)-treated polycarbonate track-etch membrane, containing 2×10^6 $3 \mu\text{m}/\text{mm}^2$ pores (Neuro Probe, USA). The exposed area of the filter was 50 mm^2 per well. The PVP-treated membrane is hydrophilic and considered to be of low adherence for cells. It was used in order to reduce the adherence and loss of migrating cells. One-fourth of lower well volume was prefilled with 100% isotonic

Percoll (GE Healthcare, Finland) in order to avoid adherence and loss of transmigrated neutrophils to the bottom of the lower well. Substances tested for chemoattraction (Table 2) were added to the lower wells. Cell culture medium RPMI 1640 supplemented with 15 mM L-glutamine, 10^{-5} U/L penicillin, 100 mg/L streptomycin, 15 mM HEPES, and 7 mM NaHCO_3 was used as a negative control.

Upper wells were filled with 200 μL of 2.5×10^6 /mL neutrophil culture suspension. Chambers were incubated for 2 hours (37°C , 5% carbon dioxide, 95% humidity).

After incubation, the suspensions of upper and lower wells were resuspended into flow cytometry tubes. Neutrophils that did not migrate remained in the upper wells. The migration rate was calculated from the total number of neutrophils harvested from the lower well and expressed as percentage of the total input of neutrophils into the upper compartment of the well.

Flow cytometry. Peripheral blood neutrophil viability and chemotaxis was analyzed using a flow cytometer (FACSCanto, BD Biosciences, USA). Evaluation was made using WinMDI software (2.9 version) (18).

Forward scatter (size) and side scatter (granularity) characteristics of neutrophils served to determine the purity of cellular suspensions and to monitor morphological changes after incubations and stimulations with tested substances.

The number of migrated neutrophils was calculated by flow cytometry using reference cells (BD Biosciences, USA) according to the manufacturer's recommendations. The amount of migrated neutrophils was expressed in percentages. The viability of neutrophils was evaluated using propidium iodide (PI) and thiazole orange-labeled cells. PI stains nuclear DNA of dead cells, while thiazole orange stains both viable and nonviable cells.

Statistical data analysis. Statistical analysis was performed using SPSS for Windows 12.0 software package. Data are presented as mean percentage of

Table 2. Substances tested for chemoattraction of peripheral blood neutrophils in vitro

Potentially chemoattractants	Preparation	Concentration (final)
Negative control	Cell culture medium (RPMI 1640) (Biological Industries, Israel)	0%
IL-8	rhIL-8 (Biological Industries, Israel) / RPMI 1640	10, 30, and 100 ng/mL
Induced sputum	Induced sputum/RPMI1640	50%
Induced sputum/IL-8	Induced sputum /RPMI1640/IL-8	50% induced sputum /10, 30, and 100 ng/mL
BAL	BAL fluid/RPMI1640	50%
BAL/IL-8	BAL fluid/RPMI1640/IL-8	50% BAL / 10, 30, and 100 ng/mL

IL-8, interleukin-8, BAL, bronchoalveolar lavage.

migrated neutrophils \pm SEM.

A descriptive statistics was used for data analysis. The differences between two study groups were evaluated comparing mean values using paired Student (*t*) test. Differences among all the study groups (more than two groups) were evaluated using Kruskal-Wallis test. Statistical significance was assumed at $P < 0.05$.

Results

IL-8 induced migration is more intensive in neutrophils of COPD patients than healthy individuals. There was a direct dependence of migration of neutrophils, isolated from blood of all study groups, on IL-8 concentration (Fig. 1). This chemokine induced a higher migration of neutrophils isolated from blood of COPD patients than from healthy nonsmokers ($P < 0.05$).

The most intensive neutrophil chemotaxis was obtained stimulating these cells with 30 and 100 ng/mL of IL-8.

Considering smoking status, only the highest IL-8 concentration (100 ng/mL) induced more intensive chemotaxis of neutrophils, isolated from COPD smokers, compared to COPD ex-smokers ($P < 0.05$). Such difference between groups of healthy individuals was obtained stimulating with lower IL-8 concentration (30 ng/mL) ($P < 0.05$). No other differences concerning smoking status were obtained.

Induced sputum and BAL fluid directly stimulate neutrophil migration. Induced sputum and BAL fluid directly induced migration of neutrophils isolated from blood of all study groups ($P < 0.05$).

Induced sputum caused a higher neutrophil chemotaxis than BAL fluid in the groups of subjects

with COPD ($P < 0.05$) (Fig. 2). A modulatory effect of induced sputum and BAL fluid on neutrophil chemotaxis did not differ in healthy individuals.

No statistically significant differences concerning smoking status were obtained.

Induced sputum with addition of IL-8 stimulates neutrophil migration, but it does not depend on IL-8 concentration. Induced sputum in combination with IL-8 (10–100 ng/mL), as well as induced sputum directly, induced neutrophil migration in all the study groups ($P < 0.05$) (Fig. 3). However, chemotaxis of neutrophils isolated from blood of COPD patients and healthy nonsmokers did not depend on additional IL-8 concentration. A direct relation between neutrophil migration and additional IL-8 was obtained only in the group of healthy smokers ($P < 0.05$).

Induced sputum in combination with additional IL-8 in most cases stimulated a higher neutrophil chemotaxis in COPD patients than in healthy individuals ($P < 0.05$).

Induced sputum in the presence of 30 ng/mL IL-8 induced a more intensive migration of neutrophils isolated from COPD smokers as compared to COPD ex-smokers and isolated from healthy smokers as compared to nonsmokers ($P < 0.05$).

BAL fluid with addition of IL-8 stimulates neutrophil migration and it depends on IL-8 concentration. BAL fluid with addition of IL-8 (10–100 ng/mL) stimulated neutrophil chemotaxis in all the study groups ($P < 0.05$) and it depended on additional IL-8 concentration ($P < 0.05$) (Fig. 4). BAL fluid with addition of 30 and 100 ng/mL IL-8 induced a more intensive migration of neutrophils isolated from blood of COPD patients than only BAL fluid directly ($P < 0.05$).

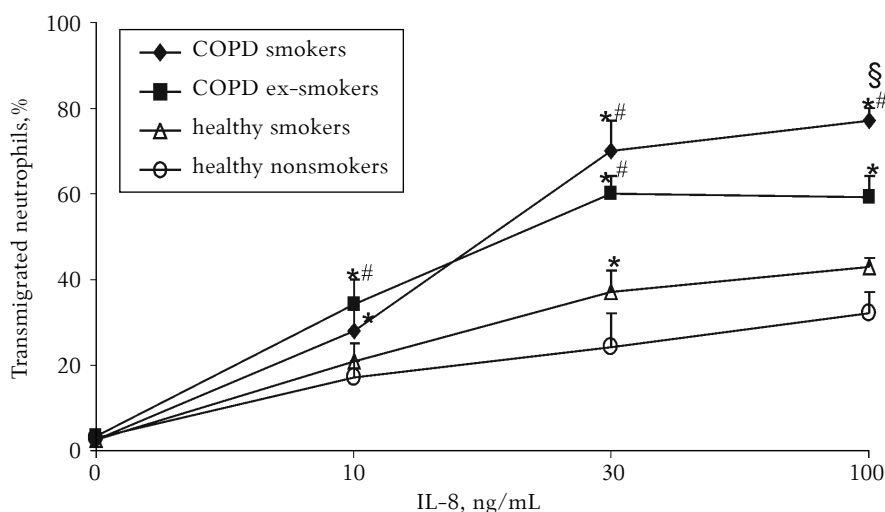


Fig. 1. The dependence of migration of peripheral blood neutrophils on interleukin-8 concentration. Data are shown as mean percentage of transmigrated neutrophils \pm SEM; * $P < 0.05$, compared to healthy nonsmokers, # $P < 0.05$, compared to healthy smokers, § $P < 0.05$, compared between COPD smokers and ex-smokers.

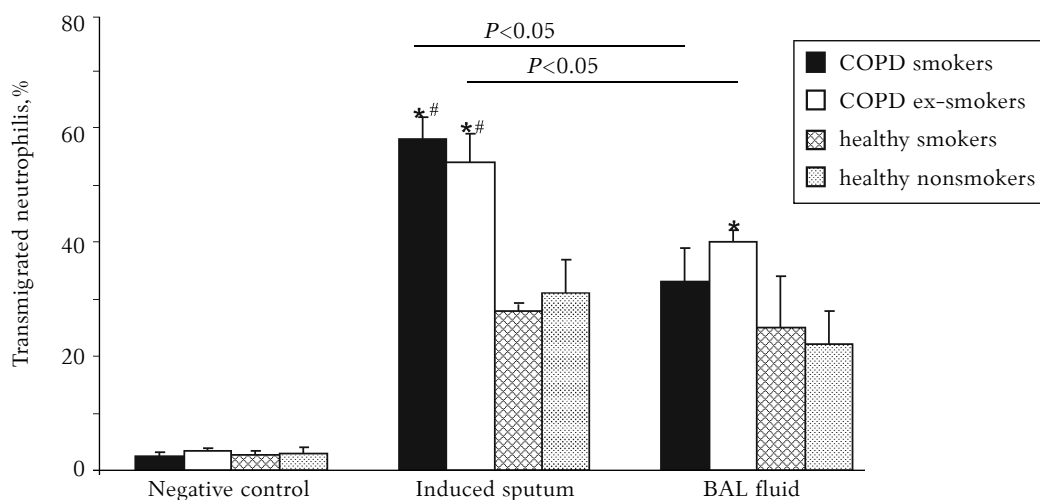


Fig. 2. The effect of induced sputum and bronchoalveolar lavage fluid on migration of peripheral blood neutrophils

Data are shown as mean percentage of transmigrated neutrophils \pm SEM;

* $P<0.05$, compared to healthy nonsmokers, # $P<0.05$, compared to healthy smokers.

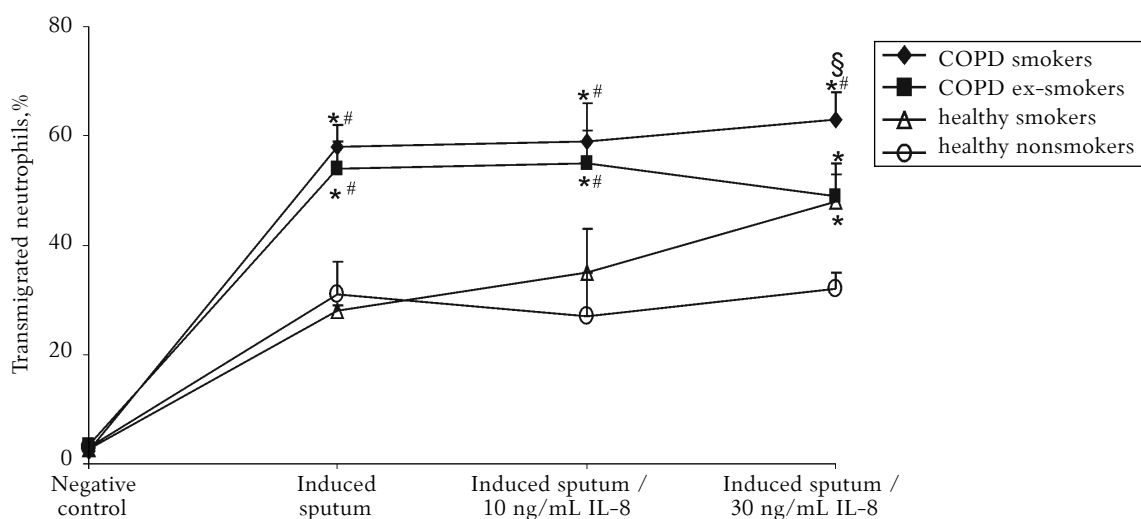


Fig. 3. The effect of induced sputum alone and in combination with different interleukin-8 concentrations on migration of peripheral blood neutrophils

Data are shown as mean percentage of transmigrated neutrophils \pm SEM; * $P<0.05$, compared to healthy nonsmokers, # $P<0.05$, compared to healthy smokers, § $P<0.05$, compared between COPD smokers and ex-smokers.

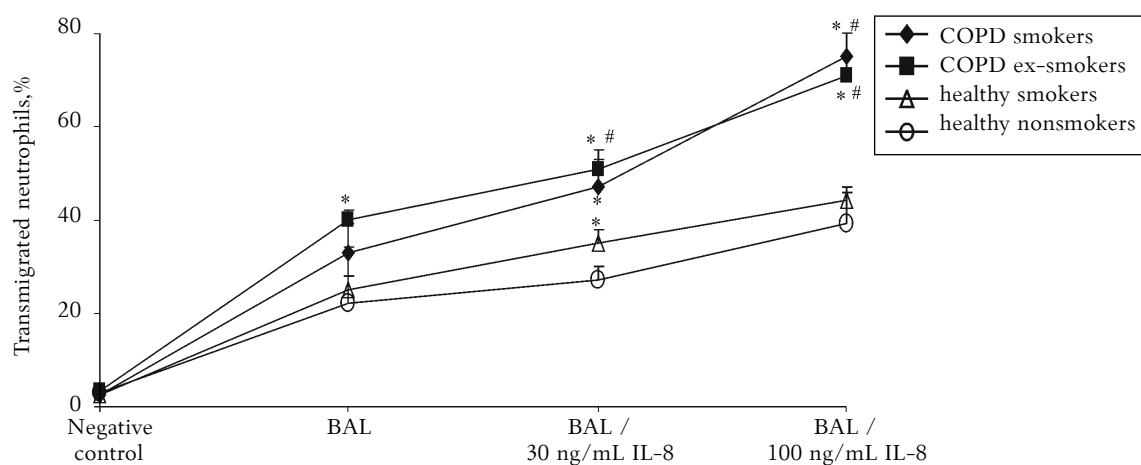


Fig. 4. The effect of bronchoalveolar lavage fluid alone and in combination with different interleukin-8 concentrations on migration of peripheral blood neutrophils

Data are shown as mean percentage of transmigrated neutrophils \pm SEM;

* $P<0.05$, compared to healthy nonsmokers, # $P<0.05$, compared to healthy smokers.

The highest neutrophil chemotaxis was registered using BAL fluid with addition of 100 ng/mL IL-8 both in the COPD groups and healthy individuals ($P<0.05$).

Concerning smoking status, no statistically significant differences in neutrophil migration were seen comparing COPD groups. Greater chemotaxis of neutrophils activated by BAL fluid with addition of 30 ng/mL IL-8 was documented in healthy smokers as compared to healthy nonsmokers ($P<0.05$).

Discussion

The study was designed to evaluate a possible chemotactic effect of induced sputum and BAL fluid from COPD patients on neutrophils isolated from peripheral blood. In addition, a possible relationship between neutrophil chemotaxis and smoking status was investigated.

Neutrophils isolated from blood of COPD patients showed a more intensive migration than those isolated from healthy individuals in the presence of IL-8. It suggests a higher reactivity and prestimulation of neutrophils isolated from patients with COPD compared to cells of healthy individuals, especially nonsmokers. Thus, increased chemotaxis of neutrophils may be determined by an increased activity of neutrophils during inflammatory process, which may stimulate the synthesis of chemoattractants and proinflammatory markers during COPD.

The differences in migration between COPD groups according to smoking status were obtained only in neutrophils activated with the highest concentration of IL-8. While, lower concentration of IL-8 was enough to observe such differences between groups of healthy individuals. It may suggest not only the prestimulation of neutrophils from COPD patients, but a decreased sensitivity to chemotactic signals as well. Neutrophils of healthy individuals react to chemotactic signal more rapidly and sensitively than those isolated from COPD patients. Neutrophils of COPD patients may be adapted to increased concentration of IL-8, which is obvious during this disease. It excludes smoking as the most significant cause of this abnormal neutrophil function and increases an importance of the disease itself.

Moreover, adenosine triphosphate energy is used for neutrophil chemotaxis (19); thus, too intensive migration of neutrophils during COPD may lead to energy depletion and other pathophysiological damages: cell hypoxia, ischemia, cytolysis, increased ROS production (2, 8). All these events may further intensify inflammation and systemic damages.

The most important finding of this study is that both induced sputum and BAL fluid stimulated neutrophil chemotaxis. A stimulating effect of these airway fluid samples may be explained by several

mechanisms. An increased concentration of IL-8 in induced sputum and BAL fluid samples of COPD patients may not account for it. According to our previous study (12), the concentration of IL-8 in induced sputum and BAL fluid of COPD patients is much lower than that needed to obtain the differences for chemotaxis in vitro.

Thus, a chemotactic effect of induced sputum and BAL fluid is modulated by other biologically active components as well. Beeh and colleagues (20) have also suggested that other components of induced sputum from COPD patients are also able to stimulate the chemotaxis of neutrophils in vitro (20). These authors have also observed that the pretreatment of neutrophils with the combination of anti-chemotactic drugs (anti-IL-8 and anti-leukotriene B₄ receptor) reduced sputum-induced chemotaxis by just 45%, thus suggesting the presence of other, yet unidentified, neutrophil chemoattractants in the sputum. Otherwise, due to a huge amount of biologically active components both in induced sputum and BAL fluid, it is difficult to identify exact chemotactic components of these samples.

It is important to note that chemotaxis of neutrophils was analyzed as in vitro model. The process of neutrophil migration in vivo occurs through a series of steps controlled and coordinated by the interaction of adhesion molecules on the surface of the neutrophil and proteins secreted by the bronchial endothelial cells (4–5, 21). Thus, a variety of intracellular interactions and a number of active mediators are lost during in vitro analysis.

Moreover, the most intensive migration of neutrophils was determined by induced sputum and BAL fluid in addition to IL-8. Such findings prove once more an importance of other components, excluding IL-8 in induced sputum and BAL fluid.

Interestingly, chemotactic role of induced sputum and BAL fluid with addition of IL-8 has also differed. The stimulating effect of induced sputum directly and with addition of IL-8 led to an intensive neutrophil migration and it did not depend on IL-8 concentration. Meanwhile, the stimulating effect of BAL fluid directly depended on additional IL-8 concentration.

Such results could be explained by several hypotheses as well. The most likely hypothesis is that induced sputum may contain more chemoattractants, especially bacteria. It is recognized that induced sputum and BAL fluid represent different compartments of airways (22). Induced sputum samples more bronchial lining fluid of the larger airways, containing higher amount of bacteria and their components that extend the survival of neutrophils ex vivo and this activity does not differ in smokers who are healthy or who have COPD, or in healthy nonsmoking subjects (22, 23), whereas BAL

fluid represents more distal airways (22) containing lower number of bacteria.

Neutrophils are predominant cell type in induced sputum (1–3), but not in BAL fluid samples. It also may suggest a high concentration of mediators responsible for neutrophil chemotaxis in induced sputum.

Another hypothesis, explaining nondiffering chemotactic effect of induced sputum on additional IL-8 concentration – the majority of sputum components may stimulate neutrophil chemotaxis through the same signaling mechanism as IL-8. Previous studies have shown that IL-8 and other members of the CXC chemokine family, chemotactic for neutrophils, act through a family of seven transmembrane domain G-protein-coupled chemokine receptors. IL-8 binds with equal affinity to 2 types of receptors expressed on neutrophils – CXCR1 and CXCR2 (24).

Thus, despite IL-8, circulating blood neutrophils migrate into the respiratory tract under the action of other components of induced sputum and BAL fluid. Quint and colleagues have suggested that neutrophil influx is also dependent on the presence of macrophage-derived matrix metalloproteinase (MMP)-12 (15). Release of MMP-12 from macrophages via an auto-feedback loop causes macrophages to release tumor necrosis factor α , resulting in neutrophil influx. Once neutrophils are beyond the endothelial

barrier, chemotactic signals lead to their accumulation near mucosal epithelial cells and in the lumen of the airway. Our previous (25) and other (26) studies have demonstrated an increased MMP-12 expression in induced sputum and BAL fluid of COPD patients, especially smokers.

Thus, there is a need to clearly identify the chemotactic factors that, among many others, may be more important in COPD patients. Many researchers suggest that neutrophil chemotaxis is the key in COPD pathogenesis (27–29), and by inhibiting or reducing the neutrophil recruitment into the airways, one should be able to reduce airway inflammation. Otherwise, other cell types (macrophages, lymphocytes, or eosinophils) are also involved in the genesis of airway inflammation.

Conclusions

Chemotaxis of neutrophils, isolated from patients with COPD, was more intensive compared to healthy individuals and depended on the concentration of chemotactic factors. Induced sputum stimulated chemotaxis of neutrophils isolated from patients with COPD as compared to healthy individuals. BAL fluid directly and with addition of IL-8 stimulated chemotaxis of neutrophils isolated from patients with COPD and healthy individuals. Migration of neutrophils did not depend on smoking status.

Sergančiųjų lėtine obstrukcine plaučių liga indukuotų skreplių ir bronchoalveolinio lavažo skysčio poveikis neutrofilų migracijai *in vitro*

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Raktažodžiai: neutrofilai, migracija, lėtinė obstrukcinė plaučių liga, indukuoti skrepliai, bronchoalveolinio lavažo skystis.

Santrauka. Tyrimo tikslas. Įvertinti galimą chemotaktinį indukuotų skreplių ir bronchoalveolinio lavažo skysčio poveikį sergančiųjų lėtine obstrukcine plaučių liga (LOPL) ir sveikų asmenų kraujo neutrofiluose.

Metodika. Į tyrimą įtraukti 43 rūkantieji ir 19 neberūkančiųjų, sergančių LOPL, 13 sveikų rūkančiųjų ir 17 sveikų nerūkančiųjų.

Neutrofilai buvo išskirti iš tiriamųjų periferinio kraujo. Siekiant užtikrinti vienodas tyrimo sąlygas, neutrofilų migracijai vertinti naudota 20 sergančiųjų LOPL indukuotų skreplių ir bronchoalveolinio lavažo skysčio sanakaupa.

Neutrofilų chemotaksis *in vitro* atliktas ląstelių transmigracijos kameroje. Apatiniai kameros šulinėliai buvo užpildomi potencialiais chemoatraktantais (interleukinu-8, indukuotais skrepliais, bronchoalveolinio lavažo skysčiu be priedų ar papildyti interleukinu-8). Viršutiniai kameros šulinėliai – $2,5 \times 10^6$ /ml neutrofilų kultūromis ir inkubuojami 2 val. Migracija vertinta tėkmės citometrijos metodu.

Rezultatai. Interleukinas-8 (10–100 ng/ml) lėmė tiesiogiai nuo koncentracijos priklausomą neutrofilų migraciją visose tirtose grupėse. Atsižvelgiant į rūkymo įprotį, tik aktyvinimas 100 ng/ml koncentracijos interleukinu-8 sukėlė intensyvesnę sergančiųjų LOPL rūkančiųjų nei neberūkančiųjų neutrofilų chemotaksį

($p < 0,05$). Sveikų asmenų grupėse tokiam skirtumui nustatyti pakako neutrofilų aktyvinimo 30 ng/ml koncentracijos interleukinu-8 ($p < 0,05$).

Indukuoti skrepliai/interleukinas-8 (10–100 ng/ml) kaip ir indukuoti skrepliai tiesiogiai skatino neutrofilų migraciją ($p < 0,05$). Sergančiųjų LOPL ir sveikų nerūkančiųjų neutrofilų chemotaksio intensyvumas nepriklausė nuo papildomos interleukino-8 koncentracijos.

Bronchoalveolinio lavažo skystis/interleukinas-8 (30–100 ng/ml) skatino intensyvesnę sergančiųjų LOPL neutrofilų migraciją nei bronchoalveolinio lavažo skystis be priedų ($p < 0,05$).

Išvados. Sergančiųjų LOPL neutrofilų migracija buvo intensyvesnė nei sveikų asmenų ir nepriklausė nuo rūkymo įpročio. Indukuoti skrepliai ir bronchoalveolinio lavažo skystis tiesiogiai ir papildytas interleukinu-8 skatino neutrofilų chemotaksį ir jis buvo intensyvesnis sergančiųjų LOPL nei sveikų asmenų neutrofiluose. Kraujo neutrofilų migracija nepriklausė nuo rūkymo įpročio.

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