Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine

A. Barczyk, W. Pierzcha³a and E. Sozańska

Department of Pneumology, Silesian Medical Academy, Katowice, Poland

Abstract

Background: Interleukin-17 (IL-17) is a novel cytokine secreted by activated human memory CD4+ T cells. In vivo IL-17 recruits neutrophils into the airways via the release of CXC chemokines (interleukin-8) from bronchial epithelial cells. Since neutrophils are implicated in pathogenesis of chronic obstructive pulmonary disease (COPD), chronic bronchitis (CB) and asthma, we hypothesized that there would be increased concentration of IL-17 in the airways of these patients. To test this hypothesis, we measured levels of IL-17 in induced sputum of COPD patients, chronic bronchitis and asthmatics and compared them with healthy controls. Methods: Levels of IL-17 in induced sputum were measured via ELISA method in 19 COPD, 16 CB, 10 asthma and 11 control subjects. Airway responsiveness to methacholine was performed in people with FEV1 higher than 70% of predicted. Results: There were no significant differences in IL-17 levels between control group and the other groups. However, levels of IL-17 in sputum of COPD patients were significantly lower than in asthma (P=0.004) and in CB (P=0.01) groups. Medians and (ranges) were as follows: asthma — 37.6 pg/ml (18.8–55.7 pg/ml), CB 29.3 pg/ml (18.8–49.7 pg/ml) and COPD 24.6 pg/ml (0–34.1 pg/ml). Comparison of healthy control subjects (PC20 > 8 mg/ml) to a group with bronchial hyperreactivity, which consisted of asthmatics and CB patients, whose PC20 was less than 8 mg/ml, revealed that levels of IL-17 were significantly increased in the second group (P=0.02). Also, levels of IL-17 were significantly increased (P=0.02) in the asthmatic patients with bronchial hyperreactivity compared to healthy subjects. Moreover levels of IL-17 in sputum of all studied subjects correlated negatively with PC20 (r = −0.51, P=0.002). Conclusions: According to our results IL-17 is probably not involved in pathogenesis of stable COPD, but it may play a role in people with airway hyperresponsiveness. © 2003 Elsevier Science Ltd. All rights reserved.


Keywords interleukin-17; asthma; COPD; airway hyperresponsiveness.

INTRODUCTION

Bronchial asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis (CB) are different chronic diseases, but inflammation of the airways is important in pathogenesis of all these conditions. Although mechanisms for development of these diseases are different, both recruitment and activation of neutrophils seems important in all these conditions (1–5). Neutrophils are capable of producing proteolytic enzymes and free oxygen radicals, which may contribute to the development of airway obstruction, especially in COPD patients but also in asthmatics (6,7). T-cells are very likely to control the recruitment of granulocytes (especially eosinophils but also neutrophils) into the airways in obstructive airway diseases. For asthma, this has been shown in animal models, but not as yet in COPD (8).

There are also some suggestions that T-cells can determine airway hyperresponsiveness (AHR) via recruitment and activation of neutrophils (9).

Interleukin-17 (IL-17) is a novel cytokine secreted only by activated CD4+ and CD8+ human memory T cells (10, 11). Both the Th1 and Th2 subsets of CD4+ cells release IL-17. However, IL-17 receptor is widely distributed and is expressed in almost all tissues, including the lung, and on several cells, including bronchial epithelial cells (12). There is increasing evidence that IL-17 is a potent cytokine mediating inflammatory responses in various tissues.

In human lung, it was shown that IL-17 induces IL-1β and TNF-α production (13). IL-17 is also important for host defence against bacteria infection. IL-17R knockout mice displayed a significant delay in neutrophil recruitment into the alveolar space after K. pneumoniae infection (14). IL-17 may indirectly recruit neutrophils into the airways via the release of mediators from other airway cells (15) (e.g. by the release interleukin-8 from bronchial epithelial cells (16) or by induction of release of endogenous tachykinins acting via NK-1 receptors (17)). Thus,
apparently, IL-17 could link activation of T-cells with recruitment and activation of neutrophils.

Since neutrophils are implicated in pathogenesis of chronic obstructive pulmonary disease (COPD), chronic bronchitis and asthma, we hypothesized that there would be increased concentration of IL-17 in the airways of these patients. To test this hypothesis, we measured levels of IL-17 in induced sputum of COPD patients, chronic bronchitis and asthmatics and compared them with healthy controls.

**MATERIAL AND METHODS**

**Subjects**

The study protocol was approved by ethics committee of the Silesian Medical Academy. All subjects gave written informed consent before they entered the study.

Nineteen patients with COPD were studied. All patients developed symptoms after the age of 40, and were current or ex-smokers with at least 10 pack-year smoking history. All patients had FEV1/VC of less than 70 percent of predicted, FEV1 of less than 85 percent of predicted and reversibility of bronchial obstruction of less than 10 percent of predicted, 15 min after 200 μg of inhaled salbutamol. Exclusion criteria were: history of atopy; exacerbation of COPD or use of systemic steroids during 4 weeks before the study. Five COPD patients were not treated. Others received conventional COPD treatment: anticholinergic agents, xanthines, short- and long-acting β2-agonists. Seven of them were additionally treated with inhaled steroids (budesonide 200–1200 μg/day).

Sixteen patients with CB had to fulfill the following criteria: presence of cough and sputum production for most days for at least 3 consecutive months during 2 consecutive years; no present or past history of other diseases of the lung (including asthma and its symptoms, such as wheezing or dyspnoea at night), which may cause identical symptoms as CB; reversibility of bronchial obstruction of less than 10 percent of predicted, 15 min after 200 μg of inhaled salbutamol (performed in subjects with FEV1 of less than 90 percent of predicted); no atopic history; no exacerbation of CB or use of systemic steroids during 4 weeks before the study. Among all chronic bronchitis patients only one was treated (anticholinergic drug and long acting β2-agonist).

Ten patients with bronchial asthma have been studied. The inclusion criteria were the following: diagnosed bronchial asthma; reversibility of bronchial obstruction 15 min after delivery of 200 μg of inhaled salbutamol of more than 12 percent (performed in subjects with FEV1 of less than 90 percent of predicted); bronchial hyperreactivity to methacholine—PC20 < 8 mg/ml (performed in subjects with FEV1 of more than 70 percent of predicted); no exacerbation of asthma or use of systemic steroids during 4 weeks before the study. Three out of 10 asthmatics recruited to the study were not treated. Five asthma patients were treated with inhaled steroids (budesonide 400–800 μg/day).

The control group consisted of 11 healthy volunteer subjects without any respiratory symptoms or diseases. The additional exclusion criteria included: abnormal spirometry; bronchial hyperreactivity to methacholine (PC20 < 8 mg/ml); history of atopy; respiratory infection during 4 weeks before the study. Neither of eleven healthy subjects received treatment.

**Lung function**

Lung function was measured with dry spirometer (MasterLab, Jaeger, Germany). Reversibility of bronchial obstruction (spirometry, before and 15 min after inhalation of 200 μg of salbutamol) was performed in asthmatics and in all COPD patients.

**Sputum induction and processing**

Sputum induction and processing was performed according a previously described method (18). First, spirometry was performed before and 15 min after inhalation of 200 μg of salbutamol. Then subjects inhaled 7 ml of 5% saline at room temperature from an ultrasonic nebulizer (Thomex MB, Medbryt, UK). After mouthwashing they were encouraged to cough sputum. If unsuccessful, they repeated (maximal twice) the above procedure and performed spirometry every time. Sputum was poured into a Petri dish and all portions that macroscopically appeared free of salivary contamination were separated from saliva using blunted forceps. This selected portion was weighed and 4 times the volume of dithiothreitol (DTT) (Gibco BRL ultra pure) were added to the sputum. The mixture was vortexed and repeatedly aspirated and then placed in a bench rocker for 20 min. After this, a volume of D-PBS (Gibco-BRL), equal to the volume of sputum and DTT were added, and mixture was rocking for 5 min. The mixture was then centrifuged at 790 g for 10 min and the supernatant was aspirated and stored in Eppendorf tubes at −70 °C for later assay. The cell pellets from sputum centrifugation were resuspended in D-PBS. Using a Neubauer hemocytometer, the following were measured: a total cell count of leukocytes, percentage of epithelial cells and cell viability (by the trypan blue exclusion method). Slides were prepared by using a Cytospin instrument (HPW 342). Two slides were stained with May–Grunwald–Giemsa stain for differential cell counts. Percentage of macrophages, neutrophils, lymphocytes and eosinophils were determined after counting of 400 leukocytes.

**Bronchial responsiveness**

At least 24 h after sputum induction, bronchial responsiveness to methacholine was performed according Sterk.
et al’s standards in healthy subjects, CB patients and asthmatics. PC_{20} was measured by inhalation of methacholine (with doubling concentrations: 0.03–25 mg/ml) delivered by an ultrasonic nebulizer (Thomex MB, Medbryt, UK) with maximal output. Each concentration was delivered for 2 min and was followed by FEV_{1} measurement. The test was stopped when there was a fall in FEV_{1} of 20 per cent compared with initial values or when the maximal concentration was inhaled. The PC_{20} value was calculated by interpolation from a line, which connected the last two dose–response points (19).

Measurement of IL-17 levels

Concentrations of IL-17 in supernatant of induced sputum were determined by ELISA method using commercially available kit (R&D systems). The detection limit of the assay was 15 pg/ml.

Control experiments

Reproducibility of the assay for IL-17 measurements was carried out with six replicates of induced sputum samples from 8 patients. To examine the effect of DTT on IL-17 assay, the spike recovery was evaluated by spiking four levels of IL-17 into induced sputum from two different patients. The concentration of DTT in induced sputum was the same as in the study (0.05%). Additionally, influence of different concentrations of DTT (0.01, 0.05, 0.1 and 0.2%) on normal and “spiked” induced sputum was checked. All measurements were performed in duplicate.

Statistical analysis

Values are expressed as median (maximal and minimal values are in brackets). Differences between the four studied groups were first analyzed using the Kruskal–Wallis test. If significant, inter-group comparisons were performed using Mann–Whitney U-test. Correlations were determined using Spearman rank correlation. P values of < 0.05 were accepted as significant.

RESULTS

The 17 persons repeated the sputum induction after the first 7 ml of solution once and the 31 persons twice. There were no differences between the four studied groups concerning these variables (P=0.13, Kruskal–Wallis test). Also, the concentrations of IL-17 did not differ between the groups of persons who repeated the induction once, twice or did not repeat it at all (P=0.44, Kruskal–Wallis test).

Demographic dates, spirometry and bronchial responsiveness results are shown in Table I.

Induced sputum of COPD patients was characterized by significantly increased number and percentage of neutrophils (respectively P=0.02 and P<0.03) and eosinophils (respectively P=0.02 and P<0.04) as compared to healthy subjects. Asthmatics in comparison with control subjects had a significantly increased number (P=0.003) and percentage (P=0.0002) of eosinophils in their induced sputa. The number and percentage of neutrophils were significantly decreased in induced sputum of asthmatics compared to COPD patients (P<0.0003 and P<0.0002, respectively) and CB patients (P<0.001 and 0.0009, respectively). The cell viability did not differ between the studied groups and, with the exception of two persons, it was higher than 65%. The characteristics of sputum of the studied population is shown in Table 2.

Levels of IL-17 in induced sputum were detected in all subjects. The Kruskal–Wallis test for levels of IL-17 in supernatant of induced sputum for all four studied groups was statistically significant (P=0.007). There were no significant differences in IL-17 levels between the control group and other groups. However, levels of IL-17 in sputum of COPD patients were significantly lower than in asthma (P<0.004) and in CB (P<0.01) groups (Fig. 1). Medians and (ranges) were as follows: asthma — 37.6 pg/ml (18.8–55.7 pg/ml), CB 29.3 pg/ml (18.8–49.7 pg/ml) and COPD 24.6 pg/ml (0–34.1 pg/ml). Comparison of healthy control subjects (PC_{20} > 8 mg/ml) to a group with bronchial hyperreactivity, which consisted of asthmatics and CB patients, whose PC_{20} was less than 8 mg/ml, revealed that levels of IL-17 were significantly increased in second group (P<0.02) (Fig. 2). Also, levels of IL-17 were significantly increased (P<0.02) in the asthmatic patients with bronchial hyperreactivity compared to healthy subjects. Moreover, levels of IL-17 in sputum of all subjects with FEV_{1} >70 percent of predicted correlated negatively with PC_{20} (r = 0.51, P=0.002) (Fig. 3). In the asthma group, the percentage of macrophages in sputum correlated negatively with levels of IL-17 (r = −0.84, P=0.003).

The reproducibility of the assay was good. The overall coefficient of variation has been calculated to be 2.4% (range 1.3–3.5%).

The average recovery of “spiked” IL-17 from induced sputum of two studied patients were 88.2 and 66.3%. The percentage of recovered IL-17 depended on the amount of “spiked” IL-17 (the more “spiked” IL-17 the higher the recovery rate). The results of “spike” experiments are shown in Table 3.

DTT decreased levels of IL-17 in supernatant of induced sputum and this effect was dose-dependent. However, the influence of DTT on levels of IL-17 was weak when used in a typical concentration (0.05%). With this concentration, the levels of IL-17 in induced sputum were decreased by DTT by approximately 20%. The results of influence of various concentrations of DTT on measurements of IL-17 in induced sputum are shown in Fig. 4.
**Table 1.** Characteristics of study subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Asthma</th>
<th>CB</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject number</td>
<td>11</td>
<td>10</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Age (years)</td>
<td>(48.0)</td>
<td>(58.0)</td>
<td>(63.5)</td>
<td>(64.0)</td>
</tr>
<tr>
<td>(24.0–72.0)</td>
<td>(270–670)</td>
<td>(440–790)</td>
<td>(480–740)</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>(10/1)</td>
<td>(3/7)</td>
<td>(1/5)</td>
<td>(1/5)</td>
</tr>
<tr>
<td>Cigarette smoking (Y/Ex/N)</td>
<td>(3/3/5)</td>
<td>(1/3/6)</td>
<td>(3/9/4)</td>
<td>(5/14/0)</td>
</tr>
<tr>
<td>FEV1 (% pred)</td>
<td>110.6</td>
<td>75.0</td>
<td>92.1</td>
<td>45.5</td>
</tr>
<tr>
<td>(101.0–142.0)</td>
<td>(64.4–111.3)</td>
<td>(73.9–1270)</td>
<td>(22.6–82.6)</td>
<td></td>
</tr>
<tr>
<td>FEV1%FVC</td>
<td>87.3</td>
<td>66.9</td>
<td>78.4</td>
<td>51.6</td>
</tr>
<tr>
<td>(78.9–98.5)</td>
<td>(54.5–88.8)</td>
<td>(65.1–879)</td>
<td>(36.1–670)</td>
<td></td>
</tr>
<tr>
<td>(\Delta)FEV1 after salbutamol (% pred)</td>
<td>n.d.</td>
<td>20.7</td>
<td>3.1</td>
<td>3.1</td>
</tr>
<tr>
<td>(13.6–34.7)</td>
<td>(0.0–97)</td>
<td>(0–8.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC20 methacholine (mg/ml)</td>
<td>&gt; 25.0</td>
<td>0.1</td>
<td>6.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>(18.6–25.0)</td>
<td>(0.07–0.38)</td>
<td>(0.4–25.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as medians and ranges; M—males, F—females; Y—current cigarette smokers, Ex—ex-smokers, N—nonsmokers; FEV1 (% pred)—forced expiratory volume in 1 s (percentage of predicted value); FVC—forced vital capacity; \(\Delta\)FEV1 after salbutamol (% pred)—reversibility of airway obstruction 15 min after delivering 200 \(\mu\)g of salbutamol expressed as percentage of predicted value; PC20 methacholine (mg/ml)—provocative concentration of methacholine causing a 20% fall in FEV1; n.d.—not done.

**Table 2.** Characteristics of induced sputum from the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Asthma</th>
<th>CB</th>
<th>COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells*</td>
<td>2.1</td>
<td>1.7</td>
<td>4.6</td>
<td>4.2</td>
</tr>
<tr>
<td>(x10^6/g of sputum)</td>
<td>(0.2–10.9)</td>
<td>(0.7–7.7)</td>
<td>(1.2–14.1)</td>
<td>(1.1–64.0)</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>81</td>
<td>82</td>
<td>85</td>
<td>87</td>
</tr>
<tr>
<td>(47–95)</td>
<td>(74–89)</td>
<td>(40–96)</td>
<td>(74–95)</td>
<td></td>
</tr>
<tr>
<td>Macrophages (%)*</td>
<td>36.0</td>
<td>33.2</td>
<td>293</td>
<td>16.9</td>
</tr>
<tr>
<td>(192–879)</td>
<td>(11.6–54.4)</td>
<td>(3.1–54.7)</td>
<td>(5.5–66.8)</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)**</td>
<td>62.0</td>
<td>33.3</td>
<td>596</td>
<td>74.0</td>
</tr>
<tr>
<td>(98–790)</td>
<td>(7.3–64.2)</td>
<td>(24.0–94.1)</td>
<td>(296–92.2)</td>
<td></td>
</tr>
<tr>
<td>Eosinophils (%)*</td>
<td>0.5</td>
<td>27.3</td>
<td>1.6</td>
<td>2.7</td>
</tr>
<tr>
<td>(0–14.2)</td>
<td>(3.0–80.0)</td>
<td>(0.2–48.0)</td>
<td>(0–33.7)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.7</td>
<td>2.5</td>
<td>2.4</td>
<td>1.2</td>
</tr>
<tr>
<td>(0.2–70)</td>
<td>(0–144)</td>
<td>(0.6–8.9</td>
<td>(0–8.9)</td>
<td></td>
</tr>
<tr>
<td>Epithelial cells (%)*</td>
<td>94</td>
<td>7.5</td>
<td>4.5</td>
<td>2.8</td>
</tr>
<tr>
<td>(0–35.0)</td>
<td>(0.8–22.7)</td>
<td>(1.3–10.7)</td>
<td>(0–17.5)</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as medians and ranges. *P < 0.05, **P < 0.0005 for differences between the four groups, Kruskal–Wallis.

**DISCUSSION**

Interleukin-17, a T-lymphocyte-related cytokine, is thought to link activation of certain T-lymphocytes with the recruitment and activation of airway neutrophils (8). Neutrophils seem to be important in pathogenesis of bronchial asthma, chronic bronchitis and especially in COPD (2,3). In COPD, and in CB patients, the number of neutrophils has been shown to be increased in induced sputum (20), in broncho-alveolar lavage (BAL) fluid (21,22) but not in bronchial mucosa (2). IL-17 recruits neutrophils into the airways indirectly, by the release of interleukin-8 from bronchial epithelial cells (16). Several studies showed that levels of interleukin-8 in induced sputum of COPD patients are increased (23,24). Based on these studies we presumed that levels of IL-17 might be increased in induced sputum of COPD patients and also in CB patients and asthmatics. However, results of our study showed that levels of IL-17 in induced sputum of COPD patients compared to healthy subjects were not increased, but in fact decreased, although it was not statistically significant. These results may suggest...
that IL-17 is probably not involved in the pathogenesis of stable COPD. However, IL-17 may play a role in triggering exacerbation of COPD (8). It is possible that levels of IL-17 may be increased even in stable COPD patients when studied in BAL or bronchial mucosa, because the results using these techniques are different to those using induced sputum (25).

Levels of IL-17 in induced sputum were not significantly increased in asthma patients (P=0.08) and CB patients (P=0.17). However when compared to COPD patients, levels of IL-17 were significantly increased in CB patients and especially in asthmatics. We speculate that it may be connected with an activation of CD4+ subset of T-lymphocytes, which are the main source of IL-17. CD4+ T-lymphocytes play a role in pathogenesis of bronchial asthma (26), in opposition to CD8+ T-lymphocytes, which are implicated in pathogenesis of COPD (27). There is also an additional explanation. As was recently shown, eosinophils from asthmatics are able to produce IL-17 (28). So, the increased number and activation of eosinophils in asthmatics may be responsible for increased concentration of IL-17 in induced sputum of these patients as compared to patients with COPD.

Airway hyperresponsiveness is a typical feature of asthma. Clinical studies have shown the relationship between activated inflammatory cells (including lymphocytes T, eosinophils and neutrophils) and airway hyperresponsiveness (29,30). Through the release of cytokines (mainly Th2 but also others), T cells can produce AHR indirectly by promoting the recruitment and subsequent activation of eosinophils and neutrophils (8,9,31). In our study we showed that levels of IL-17 were increased in asthmatics with AHR compared to the control group. Moreover, levels of this cytokine correlated positively
with a degree of AHR. These results indicate that IL-17 together with other cytokines produced by T lymphocytes may promote the development of AHR by activation and recruitment of granulocytes. However, further clinical studies are needed to confirm this hypothesis.

Negative correlation between IL-17 and sputum macrophages in asthmas could suggest some connection between them. However, we think that it reflected only the influence of IL-17 on the other cells in induced sputum (there is a positive correlation between levels of IL-17 and the sum of the percentages of neutrophils, eosinophils and lymphocytes, \( r = 0.84, P = 0.003 \)). Moreover, further analysis showed that only neutrophils and eosinophils are important for the existence of that correlation because IL-17 correlated only with the sum of neutrophils and eosinophils \((r = 0.77, P = 0.009)\), but not with the sum of lymphocytes with either eosinophils or neutrophils. We think that these results further support the potential association between IL-17 and the activation and recruitment of granulocytes in asthmatic patients.

The group of patients with chronic bronchitis may appear heterogeneous because some of them have airway hyperresponsiveness, the percentage of eosinophils in sputum is up to 48% and some of them are non-smokers. However, we think that all 16 chronic bronchitis patients were correctly diagnosed. All of them have characteristic symptoms of chronic bronchitis. Exclusion of other diseases, which could cause chronic productive cough, was based on a detailed history taken according to a specially designed protocol. Special effort was made not to qualify to the study the patients with a history of any symptoms characteristic of asthma, such as: wheezing or dyspnoea at night.

Some authors, in order to better distinguish patients with chronic bronchitis from asthmatics, do not qualify to the CB group the patients with more than 5% eosinophils in sputum or with a greater than 10–15% improvement in FEV1 after treatment with bronchodilator (32–34). In our study, we performed the bronchial reversibility test after salbutamol only in part of CB patients (with FEV1 less than 90% predicted), because we think that performing this test in patients without even slight bronchial obstruction has no diagnostic value. All CB patients who had performed a bronchial reversibility test had its value of less than 10% predicted. We did not exclude patients with sputum eosinophilia from our study. However, 15 patients had less than 4% of eosinophils in sputum. The only one patient who exceeded this limit had 48% of eosinophils in sputum. He is a life-long ex-smoker, who smoked one pack of cigarettes per day for 50 years and stopped smoking 7 years ago. His first symptoms of chronic productive cough started 5 years ago at the age of 65. His reversibility of bronchial obstruction after salbutamol was 3.2% predicted and his PC20 was 4.8 mg/ml. In our opinion his age, smoking history, no asthmatic symptoms in history, poor reversibility indicate very little probability of coexistence of asthma with chronic bronchitis. However, we also performed a statistical analysis without this patient, to check his impact on results. This analysis showed that differences are still significant (increased levels of IL-17 in CB compared to COPD \((P = 0.02)\), increased levels of IL-17 in a group with bronchial hyperreactivity compared to healthy control subjects \((P = 0.03)\) and a negative correlation between levels of IL-17 and PC20 \((r = -0.52, P = 0.002)\)).

The mean PC20 for the CB group was 10.8 mg/ml and the median was 6.1 mg/ml (range 0.4–25 mg/ml). These data indicate that part of our CB patients had mild-to-moderate airways hyperresponsiveness. However, a comparison with data from literature shows that range, distribution and mean of PC20 of CB patients in our study is typical of values seen in chronic bronchitis (32–36). Finally, although chronic bronchitis is more prevalent among smoking patients, it may also be found in non-smokers.

The percentage of neutrophils in induced sputum of normal subjects is higher in our study compared to the literature. We think that it may be a result of the influence of air pollution. Katowice is a capital of a coal-mining region, which is the most air-polluted place in Poland. Additionally, some of our healthy volunteers are retired coal-miners, who worked in coal mines for more than 20 years. Of course, some of them smoked, too. They did not have any symptoms of lung diseases. However, although the percentage of neutrophils in sputum of healthy volunteers in our study was relatively high, it was still significantly lower than in COPD group.

Our control experiments with the influence of DTT on the assay measurements showed that levels of IL-17 in induced sputum measured by ELISA method in the presence of 0.05% DTT were decreased compared to real levels, but this effect was relatively weak (approximately

![FIG. 4. Influence of different concentrations of DTT on measurements of IL-17 in 'spiked' induced sputum.](image-url)
a 20% decrease). Additionally, this effect was the same for all studied groups, so it did not have any effect on statistical analysis.

According to our results, IL-17 is probably not involved in pathogenesis of stable COPD, but it may play a role in patients with bronchial hyperreactivity.

REFERENCES


11. Shin HC, Benbernou N, Esnault S, Guenounou M. Expression of IL-


