

## Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease

A. Pesci\*, B. Balbi\*\*, M. Majori\*, G. Cacciani+, S. Bertacco\*, P. Alciato\*\*, C.F. Donner\*\*

*Inflammatory cells and mediators in bronchial lavage of patients with chronic obstructive pulmonary disease. A. Pesci, B. Balbi, M. Majori, G. Cacciani, S. Bertacco, P. Alciato, C.F. Donner. ©ERS Journals Ltd 1998.*

**ABSTRACT:** Cigarette smoking is the most important cause of chronic obstructive pulmonary disease (COPD). Although the precise sequence of events that leads a smoker to experience airway obstruction is not completely clear, airway inflammation is a relevant factor.

To investigate airway inflammation, 12 nonatopic smoking COPD patients with a forced expiratory volume in one second (FEV<sub>1</sub>)  $\delta$ 75% predicted and 10 normal non-smoking subjects (NS) were studied with bronchoscopy and bronchial lavage (BL).

Serum immunoglobulin (Ig)E levels of COPD patients correlated with the smoking history ( $r=0.7$ ,  $p=0.008$ ). In BL of COPD patients there was an increase of neutrophils (median, range) (COPD  $62.6 \times 10^3$ , 1.2–323, NS 1.35, 0–19.2,  $p=0.001$ ), eosinophils (COPD 1.6, 0–6.9, NS 0.15, 0–3.7,  $p=0.035$ ), the levels of interleukin (IL)-8 (COPD  $1079 \text{ pg}\cdot\text{mL}^{-1}$ , 121–2,500, NS 20.4, 7.2–59,  $p=0.001$ ), myeloperoxidase (MPO) (COPD  $752 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ , 11–5,500, NS 22.1, 8–70,  $p=0.001$ ) and eosinophil cationic protein (ECP) (COPD  $21.5 \text{ }\mu\text{g}\cdot\text{L}^{-1}$ , 1.8–161, NS 2, 1.8–4.9,  $p=0.001$ ). Significant correlations were found in BL of COPD patients between IL-8 and neutrophils ( $p=0.02$ ), MPO and neutrophils ( $p=0.02$ ), IL-8 and MPO ( $p=0.0001$ ) and ECP and eosinophils ( $p=0.02$ ). In addition, the ratios between the BL levels of MPO and the number of neutrophils and between ECP levels and eosinophils were higher in COPD patients than in NS ( $p=0.03$  and 0.01, respectively).

These data suggest that cigarette smoke is associated with increased amounts of airway interleukin-8, a chemotactic factor for neutrophils and eosinophils. Recruited neutrophils and eosinophils are activated and they release increased amounts of inflammatory mediators capable of damaging the bronchial tissue.

*Eur Respir J 1998; 12: 380–386.*

\*Dept of Respiratory Diseases, University of Parma, Parma, Italy. \*\*Salvatore Maugeri" Foundation, IRCCS, Rehabilitation Institute of Veruno, Italy. +Dept of Pneumology, City Hospital, Parma, Italy.

Correspondence: A. Pesci  
Istituto di Clinica delle Malattie dell'Apparato Respiratorio  
Università di Parma  
Via Rasori 10  
43100 Parma  
Italy  
Fax: 39 521 292615

Keywords: Bronchial lavage  
chronic obstructive pulmonary disease  
eosinophilic cationic protein  
interleukin-8  
myeloperoxidase

Received: November 3 1997  
Accepted after revision April 4 1998

Cigarette smoking is the main cause of chronic obstructive pulmonary disease (COPD) [1–3]. The pathogenesis of smoke-induced COPD has been evaluated in a number of studies showing the accumulation of inflammatory cells in patients' airways: predominantly polymorphonuclear leukocytes in samples representative of intraluminal inflammation (sputum, bronchial and bronchoalveolar lavage) and mainly mononuclear cells, lymphocytes and macrophages, in tissue specimens [4–13].

While the reason (or reasons) for this discrepancy is still not completely clear, these studies have opened the field to new questions. Since the relevance of airway inflammation in COPD has been demonstrated, the next step is the characterization of the different features of airway inflammation, such as the nature of chemotactic signal(s) and the contribution of the various cell types to airway inflammation through the release of inflammatory mediators.

In this regard, this study was targeted on a COPD patient population of nonatopic current smokers with airway obstruction, with the aim of investigating the characteristics of intraluminal airway inflammation in these patients. Therefore, this study evaluated the presence in

bronchial lavage (BL) of: interleukin (IL)-8, a chemotactic factor for polymorphonuclear cells into the airways; myeloperoxidase (MPO), eosinophil cationic protein (ECP) and tryptase, mediators of tissue damage from polymorphonuclear cells and mast cells; and the correlations between cellular and noncellular data obtained from BL.

### Materials and methods

#### Study subjects

Twelve patients with COPD entered this study. They all had chronic productive cough for >3 months for 2 consecutive yrs, in the absence of other known disorders, such as bronchiectasis, tuberculosis or lung cancer [14, 15]. The patients met the following inclusion criteria: 1) history of cigarette smoking, with a minimum of 15 pack-yrs, in patients who all were current smokers at time of evaluation; 2) no occupational or other exposure to other substances known to cause lung disorders; 3) absence of atopy, *i.e.* with negative skin tests for common allergen

extracts and no personal or family history of allergic disease; 4) no history of systemic or other pulmonary disease or congenital and/or acquired systemic immunodeficiency; 5) forced expiratory volume in one second (FEV<sub>1</sub>)  $\geq 75\%$  predicted and no  $>12\%$  improvement in FEV<sub>1</sub> after inhalation of 200  $\mu\text{g}$  salbutamol; 6) no bronchitic exacerbation within the preceding month; and 7) no therapy with inhaled or systemic corticosteroids and inhaled chromones within 3 months before entry into the study, but patients receiving theophylline or  $\beta$ -agonists or both were included.

As control subjects 10 normal, age-matched volunteers were studied. They all were lifetime nonsmokers, without clinical or laboratory signs of sensitization to common allergens, no history or evidence of lung disorders, serious illness or acute viral or bacterial infection in the preceding 3 months. In addition, all control subjects had normal spirometric values. Table 1 shows clinical and functional data of patients with COPD and of normal subjects.

### Study design

Before entry, patients and control subjects were screened with a clinical history, physical examination, chest radiograph and electrocardiogram. A symptom score (SS) was recorded for each COPD patient as described: three symptoms such as cough, dyspnoea, and sputum quantity were graded for severity using a 0–3 scale (0=none; 1=mild; 2=moderate; 3=severe). The day before bronchoscopy, all subjects underwent spirometric function tests (Spiroflow, Morgan, Kent, UK) and arterial blood gas evaluation (ABL Radiometer, Copenhagen, Denmark). In addition, peripheral blood was drawn from each subject and complete blood cell counts, including leukocyte differential count and serum chemistries, were performed. An aliquot of serum was frozen at  $-80^{\circ}\text{C}$ . Subsequently,

the levels of total immunoglobulin (Ig)E (PRIST; Pharmacia, Uppsala, Sweden) were evaluated. To investigate airway inflammation, fiberoptic bronchoscopy (FBS) with inspection of bronchial mucosa and all segmental bronchi and BL was performed in all subjects.

This study protocol was approved by the local ethical committee. Each subject gave informed written consent before being enrolled into the study.

### Methods

FBS was performed between 08:00 and 09:00 h on each occasion, following the guidelines of the National Institutes of Health [16]. Subjects abstained from both food and smoking for at least 12 h before FBS. Premedication consisted of nebulized salbutamol (1.25 mg) and ipratropium bromide (0.5 mg); atropine (0.5 mg) was administered by intramuscular injection 30 min before the procedure. Upper airways were anaesthetized with 4 mL of 2% lidocaine. Further lidocaine (1%) was administered through the bronchoscope channel to the lobar and/or segmental bronchi to suppress coughing. The instrument (1T10; Olympus, Tokyo, Japan) was introduced through the nose into the airways. The endoscopic bronchitis index (BI) was recorded for each COPD patient as described previously [6].

The bronchoscope was wedged into a segmental bronchus of the middle lobe and one 50 mL aliquot of sterile saline solution, warmed to  $37^{\circ}\text{C}$ , was infused. Fluid was gently aspirated immediately after the aliquot was introduced and collected in a sterile container. After recovery, BL fluid was strained through a monolayer of surgical gauze to remove mucus. The fluid was immediately centrifuged at  $500\times g$  for 10 min. Supernatants were removed and frozen in 1 mL sterile polystyrene tubes, at  $-80^{\circ}\text{C}$  until processing for mediators. The cell pellet was washed

Table 1. – Clinical and functional data of patients with chronic obstructive pulmonary disease (COPD) and normal subjects

Subject no.	Age yrs	Sex	Cigarettes smoked pack-yrs	IgE IU·mL <sup>-1</sup>	Duration of diseases yrs	Blood eosinophils n· $\mu\text{L}$	$P_{\text{a},\text{O}_2}$ mmHg	$P_{\text{a},\text{CO}_2}$ mmHg	FEV <sub>1</sub> % pred	FEF <sub>25–75</sub> % pred	PEFR % pred
<b>COPD subjects</b>											
1	69	M	50	10.4	7	120	72.2	44.7	71	63	49
2	57	M	17.5	10.9	5	410	78.4	34.8	75	68	71
3	69	M	55	49.6	9	40	70	44.6	74	82	57
4	68	M	50	51.6	9	170	65.1	45.0	74	83	66
5	55	M	40	40.8	3	170	68	38.2	75	73	56
6	89	M	40	9.6	5	330	66.6	46.3	70	54	72
7	69	M	51	60.8	4	170	65.4	49.8	75	57	76
8	67	M	50	48.9	3	240	77.0	43.8	73	89	71
9	57	M	80	90.8	3	140	78.9	34.5	60	52	43
10	48	M	16.5	11.2	5	120	71.6	40.5	61	71	41
11	63	M	24	7.5	5	80	65.3	46.0	70	57	65
12	61	F	24	1.3	20	60	72.0	45.3	75	71	67
Mean	62.6		38.6	32.8	6.5	170.8	70.8	42.7	71.08	68.3	61.2
SEM	2.03		6.7	8.1	1.4	31.5	1.4	1.3	1.5	3.5	3.4
<b>Normal subjects</b>											
Mean	59.6	7M/3F	No	ND	NA	148.7	95.5	39.6	103.12	101.2	99.6
SEM	3.5					28.8	3.8	1.1	8.2	7.6	6.8

M: male; F: female; IgE: immunoglobulin E;  $P_{\text{a},\text{O}_2}$ : arterial oxygen tension;  $P_{\text{a},\text{CO}_2}$ : arterial carbon dioxide tension; FEV<sub>1</sub>: forced expiratory volume in one second; FEF<sub>25–75</sub>: forced midexpiratory flow; PEFR: peak expiratory flow rate; ND: not done; NA: not applicable. (1 mmHg=0.133 kPa).

twice with phosphate-buffered saline solution (without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ). Cells were resuspended in Hanks' balanced salt solution and counted using a haemocytometer chamber. Cytocentrifugates (Labofuge AE, Heraeus, Germany) were stained by the May-Grünwald Giemsa method. The differential cell counts of macrophages, lymphocytes, neutrophils and eosinophils were made under light microscopy at  $\times 1000$  magnification (BH-2, Olympus), by counting approximately 300 cells in random fields. Because only a few mast cells were present and it was therefore difficult to detect them using the May-Grünwald Giemsa stain, they were counted in cell suspension after staining with toluidine blue (Sigma Chemical Co., St Louis, MO, USA), as described previously [8, 17, 18]. Mast cells, which appeared as large cells with a large nucleus and filled with red violet granules, were quantified by counting 1,000 cells. All cell data are expressed as cells·mL<sup>-1</sup> of recovered lavage fluid.

For IL-8 determinations the BL supernatants were concentrated using Centriprep concentrators (Amicon, Beverly, MA, USA) with a molecular weight cut-off of 300 Da. In brief, the fluid was placed into the sample containers of 2–3 Centriprep tubes, (10 mL fluid in each). The tubes were centrifuged at 4°C, 1500×g for 50 min. IL-8 concentrations were then determined by a two-site sandwich IL-8-specific enzyme-linked immunosorbent assay (ELISA) (Biotrak; Amersham International, Bucks, UK) [19]. This method uses mouse monoclonal antibodies (mAb) to human IL-8 coated on to the microtitre plate provided in the kit and horseradish peroxidase-conjugated goat polyclonal anti-IL-8 as the developing reagent. The concentration of IL-8 in the samples was calculated by comparison to the curve obtained with different concentrations of standards included in each kit. All samples were run in duplicate and the coefficients of variation were <10%. The minimum detectable concentration of IL-8 was 4.7 pg·mL<sup>-1</sup>.

MPO and ECP were measured in BL by a double-antibody radioimmunoassay techniques (Pharmacia Diagnostic, Uppsala, Sweden). MPO and ECP in samples compete with a fixed amount of <sup>125</sup>I-labelled MPO and ECP for the binding sites of a specific polyclonal rabbit antibodies. The techniques were carried out according to the package inserts. The interassay coefficient of variation was <6.5% for MPO [20] and 9.2% for ECP [21].

Tryptase was measured with a double-antibody radioimmunoassay (Pharmacia Diagnostic). In this assay the tryptase in BL reacts simultaneously with solid-phase anti-tryptase, bound to the test tube wall and anti-tryptase-tryptase-antitryptase <sup>125</sup>I complex. The technique was carried out according to the package instructions. The interassay coefficient of variation was <12.5% [22].

#### Data analysis

Group data were expressed as medians (range), or means  $\pm$ SEM when appropriate. Comparisons between data from bronchitic patients and from control subjects were made using the Mann-Whitney U-test. Correlations between different parameters were tested with Spearman's rank test and a p-value <0.05 was regarded as significant.

## Results

#### Clinical data

The clinical and functional characteristics of COPD patients are summarized in table 1. While no COPD patient was atopic, there was a significant correlation between the levels of total serum IgE and the smoking history ( $r=0.7$ ,  $p=0.008$ ). The total number of peripheral blood eosinophils was similar in COPD patients and in normal control subjects (eosinophils· $\mu\text{L}^{-1}$  of blood:  $170\pm 31$  in COPD patients,  $147\pm 28$  in normal subjects, NS). A symptom score was recorded from all patients (table 2). The total symptom score and the cough score correlated significantly with the number of eosinophils in the peripheral blood of COPD patients ( $r=0.7$ ,  $p=0.01$  and  $r=0.8$ ,  $p=0.002$ , respectively). All patients underwent bronchoscopic procedures without any significant complication.

#### Inflammatory mediators in bronchial lavage of COPD patients and of normal subjects

The median BL level of IL-8 in COPD patients was 1079 pg·mL<sup>-1</sup> (range 121–2,500) and in normal subjects it was 20.4 pg·mL<sup>-1</sup> (7.2–59) ( $p=0.001$ ) (fig. 1a). Similarly,

Table 2. – Endoscopic bronchitis index and symptom score of patients with chronic obstructive pulmonary disease

Patient No.	Symptom score*				Bronchitis index*				
	Cough	Sputum	Dyspnoea	Total	Erythema	Oedema	Friability	Secretion	Total
1	1	1	0	2	2	3	2	3	10
2	2	1	1	4	2	1	1	3	7
3	1	0	1	2	3	2	3	1	9
4	1	1	2	4	2	2	1	1	6
5	2	2	0	4	2	1	2	3	8
6	3	1	1	5	3	3	3	1	10
7	2	1	0	3	1	2	1	0	4
8	3	2	2	7	3	3	3	3	12
9	2	2	1	5	3	2	3	2	10
10	1	1	2	4	2	1	1	1	5
11	1	1	1	3	2	1	1	1	5
12	1	1	1	3	1	1	1	1	4

\*: score 0–3 (0=none; 1=mild; 2=moderate; 3=severe).

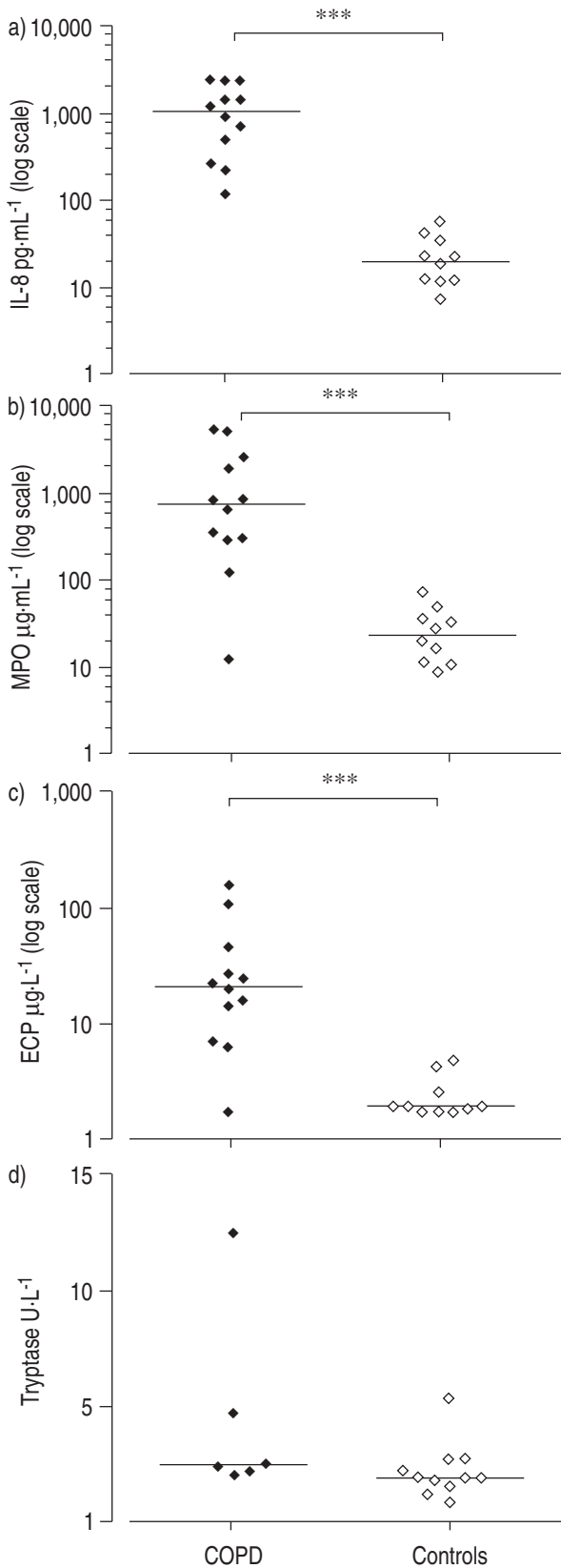


Fig. 1. – Levels of: a) interleukin (IL)-8; b) myeloperoxidase (MPO); c) eosinophil cationic protein (ECP); and d) tryptase in bronchial lavage of patients with chronic obstructive pulmonary disease and normal subjects. IL-8, MPO and ECP data are presented on a logarithmic scale. Horizontal bars represent median values. \*\*\*:  $p=0.001$ , significant difference between groups.

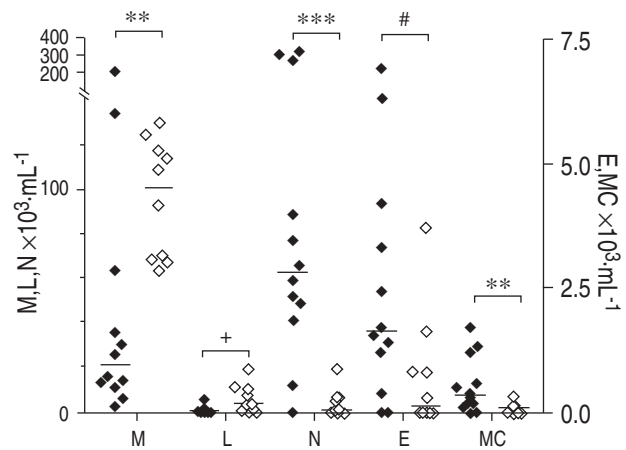


Fig. 2. – Bronchial lavage differential cell count. Data are expressed as number of different cell types·mL<sup>-1</sup> of recovered lavage fluid. ♦: chronic obstructive pulmonary disease patients; ◇: normal subjects. M: macrophages; L: lymphocytes; N: neutrophils; E: eosinophils; MC: mast cells. #:  $p=0.03$ ; +:  $p=0.02$ ; \*\*:  $p=0.01$ ; \*\*\*:  $p=0.001$ .

the median BL level of MPO in COPD patients was 752  $\mu\text{g}\cdot\text{L}^{-1}$  (range 11.5–5,500), while in normal subjects it was 22.1  $\mu\text{g}\cdot\text{mL}^{-1}$  (8–70.1) ( $p=0.001$ ) (fig. 1b) and the median BL level of ECP in COPD patients was 21.5  $\mu\text{g}\cdot\text{L}^{-1}$  (range 1.8–161.6), while in normal subjects it was 2  $\mu\text{g}\cdot\text{L}^{-1}$  (1.8–4.9) ( $p=0.001$ ) (fig. 1c).

In contrast, no significant difference was observed between the BL levels of tryptase in COPD patients and in normal subjects (fig. 1d).

*Cytological data from bronchial lavage obtained from COPD patients and normal subjects*

Analysis of cells recovered by BL in the study confirmed that bronchial inflammation in smoke-induced COPD is associated with an increase in polymorphonuclear leukocytes. In this context, BL neutrophil counts were  $62.6 \times 10^3$ , (1.2–323) in COPD patients and 1.35 (0–19.2) in normal subjects ( $p=0.001$ , fig. 2). BL eosinophil counts were  $1.6 \times 10^3$ , (0–6.9) in COPD patients and 0.15 (0–3.7) in normal subjects ( $p=0.035$ ). In addition, the number of mast cells was increased in COPD patients ( $0.35 \times 10^3$ , 0–1.7) compared with the control individuals ( $0.08 \times 10^3$ , 0–0.3,  $p=0.015$ ). Conversely, the numbers of macrophages and lymphocytes were significantly decreased in COPD patients, compared with normal subjects (fig. 2).

In an attempt to quantify the burden of mediators capable of damaging the bronchial tissue released by neutrophils and eosinophils in the airways of COPD patients and to compare this parameter with the same parameter in the airways of normal subjects, the ratios between the amounts of MPO and the number of neutrophils in BL and between the amounts of ECP and the number of eosinophils in BL were calculated. These two ratios were significantly higher in COPD patients than in normal subjects (fig. 3).

*Correlations between the levels of inflammatory mediators and cellular data*

The amounts of IL-8 in BL of COPD patients correlated significantly with the number of BL neutrophils ( $r=0.65$ ,  $p=0.02$ , fig. 4a), and with the levels of MPO ( $r=0.89$ ,

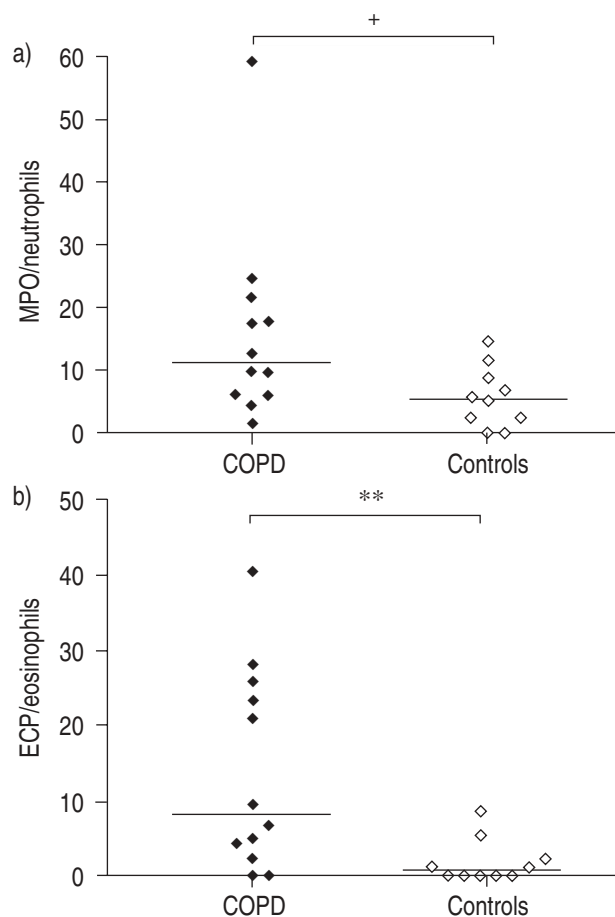


Fig. 3. – Ratios between: a) the levels of myeloperoxidase (MPO) and the number of neutrophils; and b) the levels of eosinophil cationic protein (ECP) and the number of eosinophils in bronchial lavage. Horizontal bars represent median values. +:  $p=0.03$ ; \*\*:  $p=0.01$ .

$p=0.0001$ , fig. 4b). In addition, the BL levels of the tissue-damaging effector proteins MPO and ECP significantly correlated, respectively, with the number of BL neutrophils ( $r=0.66$ ,  $p=0.02$ , fig. 4c) and with the number of eosinophils ( $r=0.64$ ,  $p=0.02$ , fig. 4d).

### Discussion

By using BL to evaluate the cellular and soluble components present in the airways of a patient population composed of current smokers with COPD, this study demonstrated: a striking increase in the amount of the chemotactic factor IL-8; an increase in inflammatory cells, mostly neutrophils, but also eosinophils and mast cells; increased levels of two inflammatory mediators, MPO and ECP, and the correlation between the levels of mediators and the number of effector cells, and also between different mediators.

Cigarette smoking is the most important cause of COPD. The presence in inhaled cigarette smoke of a number of toxic compounds is considered to be the starting point of a pathogenetic pathway in which the release of oxidants and proteases by airway and lung-resident cells, with subsequent chronic damage to the bronchial and alveolar structures, together with peribronchial inflammation and hyperplasia of mucus-secreting cells, represent the counterpart of the clinical syndrome known as COPD.

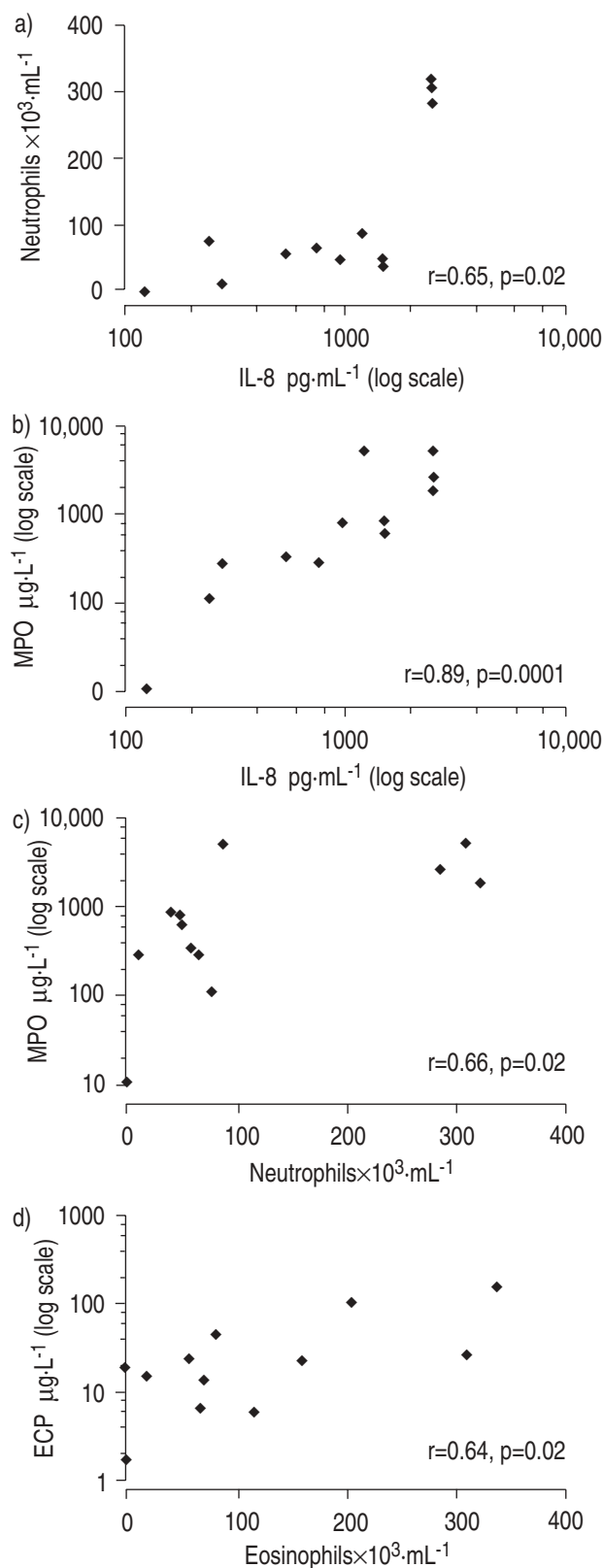


Fig. 4. – Correlations between: a) the levels of interleukin (IL)-8 and the number of neutrophils; b) the levels of IL-8 and the levels of myeloperoxidase (MPO); c) the levels of MPO and the numbers of neutrophils; and d) the levels of eosinophil cationic protein (ECP) and the numbers of eosinophils in bronchial lavage of patients with chronic obstructive pulmonary disease. IL-8, MPO and ECP data are presented on a logarithmic scale.

The precise sequence of events that leads a smoker to develop airway obstruction is not completely clear, nor are the cause(s) that, given a similar smoking history, make some individuals develop only bronchial inflammation without airway obstruction (simple chronic bronchitis), while others develop bronchial inflammation with significant airway obstruction (COPD). The link between cigarette smoke, bronchial inflammation and bronchial obstruction is relevant to understanding fully the pathogenesis of COPD in smokers.

Therefore, in these patients, IL-8 was evaluated as a possible candidate to link between cigarette smoke and airway inflammation and a striking increase in IL-8 levels was found. Similar data were reported by RUISE *et al.* [23] who evaluated, using a low-volume BL, a population of patients with chronic bronchitis. However, this study did not include any data on the cellular content of BL. In contrast, in the present study, when mediator and cellular data from BL of COPD patients were correlated, a highly significant correlation was observed between the amount of IL-8 and the number of neutrophils, although the analysis of data, as seen in figures showing the data as individual points, seems to suggest that there may be subgroups of patients with more severe inflammation ongoing in the airways.

IL-8 is a C-X-C chemokine that can be produced in the airways and in the lung by a variety of resident cells and by mobile cells which is able to recruit and activate neutrophils and, albeit to a lesser extent, eosinophils [24]. Although it is possible that smoke may increase the amounts of other chemokines or other chemotactic factors in the lung, this study demonstrates in a COPD patient population a link between the presence of increased amounts of a chemokine able to recruit granulocytes at sites of disease and the number of neutrophils crowding the airways of COPD patients. In this regard, KEATINGS *et al.* [25] showed similar correlations in the sputum from COPD patients and other researchers have shown similar data from asthmatic patients [26].

Recently, MIO *et al.* [27] reported the presence of increased amounts of IL-8 and a correlation between the levels of IL-8 and the neutrophil counts in bronchial samples of BAL from normal smokers. Thus, at least some of the changes observed in lavage samples from "normal smokers" are similar to those found in COPD patients. Although it is possible that "normal smokers" may share some features of airway inflammation with COPD patients, the present study was designed to characterize the inflammatory cells and mediators in BL of current smokers with COPD, rather than to compare smokers with disease to smokers without clinically evident disease.

It has already been demonstrated that polymorphonuclear cells (PMN) are selectively increased in lavage samples from COPD patients, both in bronchoalveolar lavage (BAL) and in BL [4–8]. In this context, the present data show that neutrophils are present in BL of COPD patients in proportions varying among different patients, from  $10^{-10}$  and eosinophils from  $10^{-10}$  orders of magnitude greater than those observed in BL of normal subjects.

If neutrophils are the characteristic cells in lavage samples from COPD patients, eosinophils are expected to be found in increased proportions mainly in BL or BAL from asthmatic patients [7]. Still, in the present COPD patients, selected as nonatopic and current smokers, increased numbers of BL eosinophils, increased levels of ECP and a

correlation between them was found. The data on ECP in lavage from COPD patients are not consistent in the literature, although the different results may be due to different techniques of lavage: from BL (this study and Ref. [23]) to BAL [7]. Since the effect of IL-8 as a chemotactic factor is mainly on neutrophils, the increase in eosinophils in BL may simply be seen as a side-effect phenomenon. Alternatively, given the increase in capillary leakage due to airway inflammation, allergens may have easier access through the airways and this can elicit an "allergic" response with recruitment of eosinophils, as the increase of circulating IgE in smokers might suggest [28, 29]. Another possibility is that, in the present study, the patient selection, although it was made using the internationally accepted criteria, may include some patients with an intrinsic asthmatic background relevant to the airway inflammation.

The data collected in this study population therefore confirm the previous observations on the increase in BL PMN and add the finding of increased numbers of BL mast cells. Mast cells were found in increased proportions in the first aliquots of BAL performed by LUSUARDI *et al.* [30] in a selected population of patients with chronic bronchitis who never smoked while, in another patient population, comprising mostly lifetime nonsmokers or former smokers, no significant increase of the proportions of mast cells was found [8]. Evaluating bronchial biopsies showed that mast cells are increased in bronchial submucosal glands in patients with chronic bronchitis, and that the mast cells are degranulated [18]. However, the determination in BL of the levels of tryptase, a mediator released by mast cells when they degranulate in response to an activating signal (*e.g.* IgE cross-bridging in atopic subjects), may suggest that if mast cells are increased in BL, they do not seem to play a role in intraluminal inflammation, while further studies are needed to evaluate their activities in the bronchial tissue.

If IL-8 is the, or at least one of the chemotactic factors responsible for the accumulation of increased numbers of PMN in the airways of smokers with COPD, the next question is: what are these cells doing there? The present data show that two of the main inflammatory mediators released by PMN when they are activated, *i.e.* MPO by neutrophils and ECP by eosinophils, are significantly and strikingly increased in BL from COPD patients, with clear correlations between MPO and neutrophils and ECP and eosinophils. Furthermore, the significant correlation between IL-8 and MPO seems to confirm the pathogenetic loop of recruitment into the airways and of activation of PMN in these patients. When the ratios between the inflammatory mediators (MPO and ECP) and the effector cells (neutrophils and eosinophils) were calculated and compared to those obtained in normal subjects, it was clear that in smokers with COPD, PMN are not only recruited into the airways but also activated to release their mediators that are able to damage the bronchial tissue and the airways.

In conclusion, although caution is needed in interpreting data from airway lavage, as proof of cause and effect, the present data, together with the existing evidence, seem to suggest a pathogenetic loop, comprising cigarette smoke, chemotactic factor(s), and cell and soluble mediators of tissue inflammation and damage, as the mechanism underlying airway inflammation in current smokers with COPD.

Further studies are needed in different chronic obstructive pulmonary disease patient populations (e.g. with different smoking history or habits) to define better the different profiles of inflammatory mechanisms ongoing in the airways of these patients. Understanding the pathogenetic mechanisms of chronic bronchial inflammation and obstruction at a molecular level may be relevant in the future for identifying possible targets for treatment of patients with chronic obstructive pulmonary disease.

### References

- Fletcher C, Peto R. The natural history of chronic airflow obstruction. *BMJ* 1977; i: 1645–1648.
- Sherrill DL, Lebowitz MD, Knudson RJ, Burrows B. Smoking and symptom effects on the curves of lung function growth and decline. *Am Rev Respir Dis* 1991; 144: 17–22.
- Sherrill DL, Holberg CJ, Enright PL, Lebowitz MD, Burrows B. Longitudinal analysis of the effects of smoking onset and cessation on pulmonary function. *Am J Respir Crit Care Med* 1994; 149: 591–597.
- Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction: accumulation of neutrophils in the lung of cigarette smokers. *Am Rev Respir Dis* 1983; 128: 833–838.
- Martin TR, Raghu G, Maunder RJ, Springmeyer SC. The effects of chronic bronchitis and chronic airflow obstruction on lung cell populations recovered by bronchoalveolar lavage. *Am Rev Respir Dis* 1985; 132: 254–260.
- Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis: characterization and correlation with clinical parameter. *Am Rev Respir Dis* 1989; 140: 1527–1537.
- Lacoste JY, Bousquet J, Chanez P, et al. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1993; 92: 537–548.
- Balbi B, Aufiero A, Pesci A, et al. Lower respiratory tract inflammation in chronic bronchitis. Evaluation by bronchoalveolar lavage and changes associated with treatment with Immucytal, a biological response modifier. *Chest* 1994; 106: 819–826.
- Fournier M, Lebargy F, Le Roy Ladurie F, Lenormand E, Pariente R. Intraepithelial T-lymphocyte subsets in the airways of normal subjects and of patients with chronic bronchitis. *Am Rev Respir Dis* 1989; 140: 737–742.
- Ollerenshaw SL, Woolcock AJ. Characteristics of the inflammation in biopsies from large airways of subjects with chronic airflow limitation. *Am Rev Respir Dis* 1992; 145: 922–927.
- Saetta M, Di Stefano A, Maestrelli P, et al. Activated T-lymphocytes and macrophages in bronchial mucosa of subjects with chronic bronchitis. *Am Rev Respir Dis* 1993; 147: 301–306.
- Di Stefano A, Turato G, Maestrelli P, et al. Airflow limitation in chronic bronchitis is associated with T-lymphocyte and macrophage infiltration in the bronchial mucosa. *Am J Respir Crit Care Med* 1996; 153: 629–632.
- O'Shaughnessy TC, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of CD8<sup>+</sup> T lymphocyte with FEV<sub>1</sub>. *Am J Respir Crit Care Med* 1997; 155: 852–857.
- American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995; 152: S77–S120.
- European Respiratory Society. Consensus statement: optimal assessment and management of chronic obstructive pulmonary disease. *Eur Respir J* 1995; 8: 1398–1420.
- National Institutes of Health. Workshop summary and guidelines: investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airways disease. *J Allergy Clin Immunol* 1991; 88: 808–814.
- Balbi B, Bason C, Balleari E, et al. Increased bronchoalveolar granulocytes and granulocyte/macrophage colony-stimulating factor during exacerbations of chronic bronchitis. *Eur Respir J* 1997; 10: 846–850.
- Pesci A, Rossi GA, Bertorelli G, Aufiero A, Zanon P, Olivieri D. Mast cells in the airway lumen and bronchial mucosa of patients with chronic bronchitis. *Am J Respir Crit Care Med* 1994; 149: 1311–1316.
- Boutten A, Dehoux MS, Seta N, et al. Compartmentalized IL-8 and elastase release within the human lung in unilateral pneumonia. *Am J Respir Crit Care Med* 1996; 153: 336–342.
- Bousquet J, Chanez P, Lacoste JY, et al. Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of asthmatic patients. *J Allergy Clin Immunol* 1991; 88: 449–460.
- Venge P, Roxin LE, Olsson I. Radioimmunoassay of human eosinophil cationic protein. *Br J Haematol* 1977; 37: 331–335.
- Wenzel S, Irani M, Sanders MA, Bradford TR, Schwartz LB. Immunoassay of tryptase from human mast cells. *J Immunol Meth* 1986; 86: 139–142.
- Riise GC, Ahlstedt S, Larsson S, et al. Bronchial inflammation in chronic bronchitis assessed by measurement of cell products in bronchial lavage fluid. *Thorax* 1995; 50: 360–365.
- Baggiolini M, Dewald B, Moser B. Interleukin-8 and related cytokines - CXC and CC chemokines. *Adv Immunol* 1994; 55: 97–179.
- 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: 530–534.
- Nocker RET, Schoonbrood DFM, van der Graaf EA, et al. Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. *Int Arch Allergy Immunol* 1996; 109: 183–191.
- 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: 1770–1776.
- Burrows B, Halonen M, Barbee RA, Lebowitz MD. The relationship of serum immunoglobulin E to cigarette smoking. *Am Rev Respir Dis* 1981; 124: 523–525.
- Ericsson CH, Svartengren M, Mossberg B, Camner P. Bronchial reactivity, lung function and serum immunoglobulin E in smoking-discordant monozygotic twins. *Am Rev Respir Dis* 1993; 147: 296–300.
- Lusuardi M, Capelli A, Cerutti CG, Spada EL, Donner CF. Airways inflammation in subjects with chronic bronchitis who have never smoked. *Thorax* 1994; 49: 1211–1216.