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Increased bronchoalveolar granulocytes and granulocyte/macrophage colony-stimulating factor during exacerbations of chronic bronchitis

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ABSTRACT: Although inflammatory changes are found throughout the airways of patients with chronic bronchitis, the mechanisms of the pathogenesis of chronic bronchitis are still unclear. The aim of this study was to investigate airways inflammation in patients with and without an exacerbation of bronchitis.

Thirteen chronic bronchitic patients and nine normal subjects were studied. Eight of the patients were studied under baseline conditions (B), and five during an exacerbation of bronchitis (E). Bronchoscopy and bronchoalveolar lavage (BAL) with cytological analysis were performed, and the levels of granulocyte/macrophage colony-stimulating factor (GM-CSF) were determined in sera and in BAL supernatants by a solid phase enzyme immunoassay.

Compared with patients under baseline conditions, chronic bronchitic patients with an exacerbation had increased numbers of BAL neutrophils (10±3 and 83±18× 10^3 cells·mL^{-1}, respectively; p<0.0001) and of BAL eosinophils (1.9±0.5 and 6.7±1.9×10^3 cells·mL^{-1}, respectively; p=0.014). Patients with chronic bronchitis, as a whole, had significantly increased levels of BAL GM-CSF compared to control subjects (36±5 and 19±4 pg·mL^{-1}, respectively; p=0.035), and similar levels of serum GM-CSF. Serum levels of GM-CSF were markedly increased in chronic bronchitic patients with an exacerbation, as compared with patients under baseline conditions (1.4±0.4 and 13±1 pg·mL^{-1}, respectively; p <0.0001). BAL levels of GM-CSF were also increased in chronic bronchitic patients with an exacerbation (25±5 and 54±8 pg·mL^{-1}, respectively; p=0.009).

During exacerbations of chronic bronchitis there are changes in the cell populations in bronchoalveolar lavage of patients consistent with a recruitment of polymorphonuclear leucocytes in the airway lumen. The increased levels of granulocyte/macrophage colony-stimulating factor might suggest a role for this cytokine in the inflammatory processes of chronic bronchitis.

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Chronic bronchitis is characterized clinically by chronic cough, sputum production, and recurrent exacerbations with worsening of respiratory symptoms [1–6]. Although chronic airways inflammation plays an important role in the development of progressive impairment of respiratory function, the mechanisms of the pathogenesis and the identification of the cells and mediators acting as inflammatory agents in the lower respiratory tract of patients with chronic bronchitis are still poorly understood. The pathogenesis of exacerbations of bronchitis is even more unclear. Infectious and noninfectious stimuli are thought to be the cause of these clinical events, but it is not a simple matter to differentiate between the various causes [1–3, 7].

The aim of the present preliminary study was to investigate the inflammation in the lower respiratory tract of patients with chronic bronchitis during an exacerbation, and to compare these data with those obtained from patients without an exacerbation.

Materials and methods

Bronchoalveolar lavage (BAL) cells and the levels of serum and BAL granulocyte/macrophage colony-stimulating factor (GM-CSF) were evaluated in 13 nonatopic patients with chronic bronchitis [2, 3]. Most patients were receiving theophylline and/or beta-agonists, but no patient had received therapy with agents able to act on the immune parameters within 3 months of entry into the study.

Eight of the patients were studied under baseline conditions, *i.e.* at least 6 months after the last exacerbation of bronchitis. Five patients were studied during an exacerbation of bronchitis. Therefore, the data collected in this study come from two groups of chronic bronchitic patients examined during different clinical settings.

Exacerbations of chronic bronchitis were defined as increased respiratory symptoms, mainly cough with increased (nonpurulent) sputum production and dyspnoea,

that made the patient seek medical attention, as described previously [2, 3, 8–11].

All patients of both groups had a history of cigarette smoking: four patients, two stable and two exacerbated, were current smokers, and the other patients were ex-smokers. Table 1 presents the clinical and spirometric data of the patient populations.

As control subjects, nine normal individuals (6 males and 3 females, 53±5 yrs of age) (mean±SEM) were studied. None of the control subjects had a history of atopy, or a history or evidence of lung disorders. Two control individuals had a history of cigarette smoking. One was a current smoker and the other was an ex-smoker. All control subjects had normal chest radiographs and normal lung function tests (forced expiratory volume in one second (FEV_1) 102±5% of predicted value) (median±SD).

Informed consent to all the procedures of the study was obtained from all subjects. Endoscopic procedures were performed following the guidelines for patients with obstructive airways disorders [12].

Fibreoptic bronchoscopies were performed using a flexible fibreoptic bronchoscope (Olympus BF; Olympus Co., Tokyo, Japan). After premedication with atropine (0.5–1 mg) and diazepam (5 mg), the instrument was introduced transorally or transnasally and passed through the larynx. BAL was performed by instilling 100 mL of sterile saline solution, in five 20 mL aliquots, through the fibreoptic bronchoscope wedged into the lobe selected for lavage, as described previously [13, 14]. The lavage fluid recovered was then filtered and cells were separated from the fluid by centrifugation (500×g for 5 min). Supernatants were then collected and stored in aliquots at -80°C. Total and differential cell counts were obtained as described previously [13, 14].

The concentrations of GM-CSF in sera and in BAL supernatants were determined by a specific solid phase enzyme immunoassay (EASIA; Medgenix Diagnostics, Fleurus, Belgium) as described previously [15, 16]. Concentrations as low as 3 $pg \cdot mL^{-1}$ are detectable with this assay, and cross-reactions with other cytokines have been shown to be insignificant.

Data are expressed as mean±SEM. For statistical analysis, levels of GM-CSF that fell under the level of detectability of the assay were arbitrarily considered as 1. Statistical analysis was performed using Student's t-test and the Mann-Whitney U-test. A p-value less than 0.05 was regarded as significant.

Results

No difference was found between the two groups with regard to age, smoking history, disease duration and values of FEV_1, although, patients with an exacerbation tended to have higher numbers of circulating neutrophils (p=0.05). Differential BAL cell counts showed that during an exacerbation of chronic bronchitis the cytological picture observed in the lower respiratory tract of these patients was markedly changed as compared to chronic bronchitic patients under baseline conditions. Patients with an exacerbation of bronchitis had significantly increased numbers of total BAL cells (343±51 10^3 $cells \cdot mL^{-1}$) as compared with the control group (175± 9 cells 10^3 $cells \cdot mL^{-1;}$ p=0.001) and also with chronic bronchitic patients examined under baseline conditions (218±18×10^3 $cells \cdot mL^{-1}$; p=0.021) (not shown). In addition, in the patients examined during an exacerbation, BAL neutrophils were 83±18×10^3 $cells \cdot mL^{-1}$, and BAL eosinophils 6.7±1.9×10^3 $cells \cdot mL^{-1}$, while in patients examined under baseline conditions BAL neutrophils were 10±3×10^3 $cells \cdot mL^{-1}$, and BAL eosinophils 1.9±0.5×10^3 $cells \cdot mL^{-1}$ (p< 0.0001 and p=0.014, respectively; fig. 1).

When considering chronic bronchitic patients as a whole, no significant difference was found for the serum levels of GM-CSF as compared to control subjects (5.9±1.8 and 1.5±0.5 $pg \cdot mL^{-1}$, respectively; p=0.074), while in BAL, chronic bronchitis patients had higher levels of the cytokine compared to control subjects (36±5 and 19±4 $pg \cdot mL^{-1}$, respectively; p=0.035) (not shown). Among chronic bronchitic patients, serum levels of GM-CSF were significantly higher in patients with an exacerbation than those found in patients examined under baseline conditions (13±1 and 1.4±0.4 $pg \cdot mL^{1}$, respectively; p<0.0001) (fig. 2). Moreover, BAL levels of GM-CSF were also higher in patients with an exacerbation (54±8 $pg \cdot mL^{-1}$) than in patients in baseline conditions (25±5 $pg \cdot mL^{-1}$; p=0.009) (fig. 2).

Table 1. – Characteristics of patients with chronic bronchitis under baseline conditions and during an exacerbation

Ss No.	Age	Sex	Smoking	Disease duration	Cell count $\times 10^3$ $cells \cdot \mu L^{-1}$ blood		FEV_1
	yrs		pack/yrs	yrs	Neu	Eos	% pred
Baseline							
1	64	M	42	28	2.5	0.32	65
2	72	M	49	30	2.3	0.51	70
3	52	F	35	12	3.5	0.15	73
4	72	M	30	18	2.7	0.60	86
5	55	M	43	10	2.4	0.12	67
6	38	M	20	4	4.4	0.23	72
7	57	M	12	13	1.8	0.31	61
8	48	F	36	13	2.6	0.40	92
Mean	57		33	16	2.8	0.3	71
SEM	4		4	3	0.3	0.1	10
Exacerbation							
9	73	F	56	21	5.8	0.34	52
10	65	M	33	14	2.5	0.40	70
11	56	M	46	18	6.1	0.80	58
12	60	M	28	20	2.9	0.18	64
13	54	M	34	14	4.0	0.61	67
Mean	61		39	17	4.3	0.5	64
SEM	3		5	1	0.7	0.1	7
p-value	NS		NS	NS	0.05	NS	NS

None of the patients studied during an exacerbation had >38°C of temperature or production of purulent sputum and all microbiological cultures of sputum were negative. None of the patients from the exacerbation group had evidence of pneumonia or radiographic abnormalities in the area lavaged. No patient had evidence of other causes of acute exacerbation, defined as secondary by the European Respiratory Society, such as heart failure, arrhythmias, pneumothorax, *etc*. [3]. Pulmonary function tests were performed 3–5 days after the beginning of the exacerbation. Ss: subjects; M: male; F: female; Neu: neutrophils; Eos: eosinophils; FEV_1: forced expiratory volume in one second; % pred: percentage of predicted value; NS: nonsignificant.

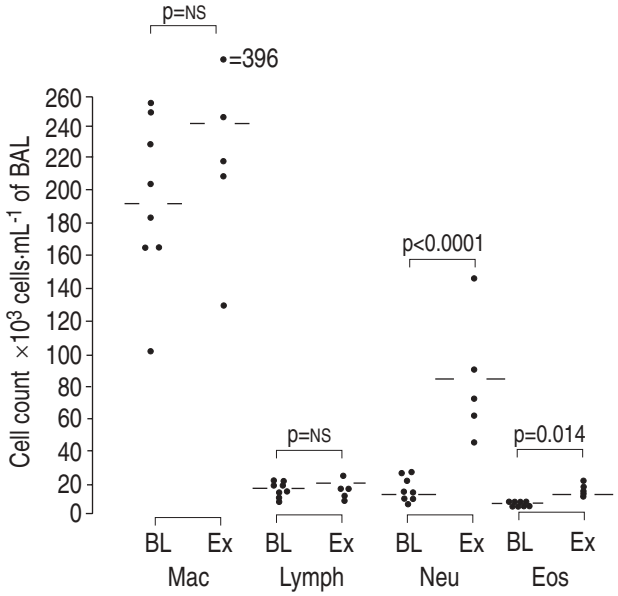

Fig. 1. – Numbers of different cell types in bronchoalveolar lavage (BAL) from patients with chronic bronchitis under baseline conditions (BL), and from patients with chronic bronchitis during an exacerbation of bronchitis (Ex). Mac: macrophages; Lymph: lymphocytes; Neu: neutrophils; Eos: eosinophils. Horizontal bars represent the mean.

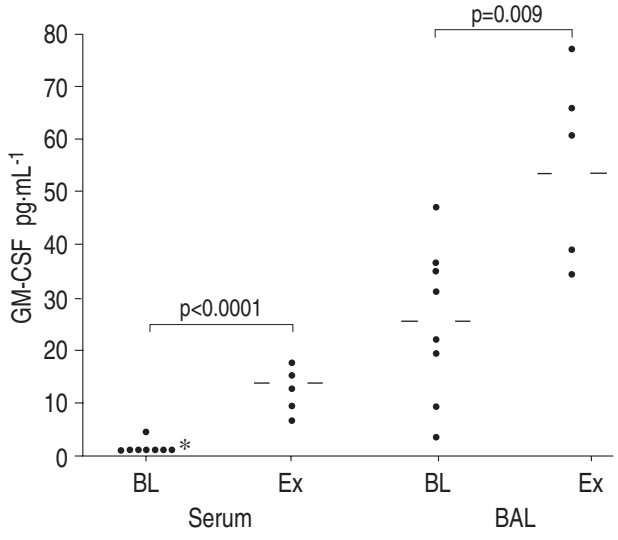

Fig. 2. – Levels of granulocyte/macrophage colony-simulating factor (GM-CSF) in serum and bronchoalveolar lavage (BAL) supernatant from patients with chronic bronchitis under baseline conditions (BL) and from patients with chronic bronchitis during an exacerbation of bronchitis (Ex). *: undetectable. Horizontal bars represent the mean.

Discussion

This preliminary study in a limited number of chronic bronchitic patients shows that the BAL cytological picture present in patients during an exacerbation is markedly different to that observed under baseline conditions, with increased numbers of total BAL cells and of polymorphonuclear leucocytes (PMNs). In addition, the serum and BAL levels of GM-CSF are also increased during an exacerbation.

Recurrent episodes characterized by increased cough with sputum production and associated increased dyspnoea are key features in the natural history of chronic bronchitis. Exacerbations of bronchitis represent one of the most relevant causes of hospitalization of patients with chronic bronchitis, with high social and economic costs, and, at the same time, probably one of the most important factors in the progression of the disease [1–7]. Nevertheless, the precise sequence of cause-effect phenomena of exacerbations of bronchitis is still under debate. Infectious and noninfectious agents are probably involved in the pathogenesis of these exacerbations. Neutrophils, and to a lesser degree eosinophils, are consistently increased in bronchial and bronchoalveolar lavage fluid of patients with chronic bronchitis and with a history of cigarette smoking [8, 17–19], and also in patients with chronic bronchitis who have never smoked [20], and this increase is correlated with smoking history and bronchial obstruction. In contrast, histological analysis of bronchial biopsies shows that the predominant cells infiltrating the bronchi of patients with chronic bronchitis are mononuclear cells. Macrophages, plasma cells and activated T-lymphocytes are found in increased proportions in the bronchial mucosa [21–24]. The studies reported above, however, have dealt with patients examined during baseline conditions. In an elegant study, SAETTA *et al*. [9] evaluated patients with chronic bronchitis during an exacerbation, defined as increased respiratory symptoms, and demonstrated an increase of eosinophils in the bronchial mucosa of nonatopic patients with chronic bronchitis.

In the present preliminary study, evaluating two small groups of chronic bronchitic patients, it was found that, during an exacerbation of bronchitis, the BAL cell number was increased, with augmented numbers of neutrophils and eosinophils, as compared to chronic bronchitic patients without an exacerbation. Although caution is needed when interpreting data coming from limited groups of patients, these observations suggest that there may be substantial changes in the airway cellular content during the phase of exacerbation. Since BAL is able to sample cells and solutes both from the bronchi and from the lower respiratory tract, it is possible that the cell changes observed in BAL from chronic bronchitic patients during an exacerbation come from both compartments. The data collected in this study, however, do not allow a distinction between changes occurring in the large airways from those taking place in the lower respiratory tract. More detailed studies are needed to try to answer this question.

Much less information is available regarding the cytokines and the inflammatory mediators released by the cells involved in the inflammation of chronic bronchitis. LINDEN *et al*. [25] found increased levels of bronchoalveolar myeloperoxidase (MPO) and of eosinophilic cationic protein (ECP) in chronic bronchitic patients, correlating with the degree of bronchial obstruction. DI STEFANO *et al*. [26] examined the number of tumour necrosis factor-α (TNF-α) and interleukin 1β (IL-1β) reactive cells in the bronchial submucosa of chronic bronchitic patients without an exacerbation, and found no significant difference compared to asymptomatic smokers and control individuals. However, during an exacerbation of bronchitis, the number of TNF-α reactive cells in bronchial biopsies was increased [9]. More recently, O'SHAUGHNESSY *et al*. [27] reported increased levels of GM-CSF reactive cells in bronchial biopsies from chronic bronchitis

patients correlating with the degree of airway obstruction.

The demonstration that GM-CSF serum and BAL levels are increased in patients with chronic bronchitis during an exacerbation is in keeping with these data. This cytokine, in addition to its known effects on stem cells for granulocytes and macrophages, is able to act on mature macrophages, neutrophils and eosinophils [17, 28–34]. A variety of cells present in the airways and in the lung parenchyma are able to produce GM-CSF, including T-lymphocytes, macrophages, and other cell types. As a proinflammatory cytokine, there is evidence of increased expression of GM-CSF in the bronchial epithelium of asthmatics, and increased levels of BAL GM-CSF have been found after bronchial allergen challenge [35–38]. In this context, the presence of increased levels of GM-CSF during exacerbations of chronic bronchitis may shed light on the pattern of cytokines in the lower respiratory tract in chronic bronchitic patients.

Following the description of two separate profiles of cytokine production from two subsets of T-cells (the type-1 T-helper (Th-1) and the type-2 T-helper (Th-2) subsets) [39], the evidence of increased levels of interleukin-4 and -5 (IL-4 and IL-5) in samples from asthmatic patients have suggested that there might be a Th-2 cytokine profile in these patients [38, 40]. Other evidence supports the hypothesis that in patients with chronic obstructive pulmonary disease (COPD), T-lymphocytes present in the lower respiratory tract might be of the Th-1 subset [41]. However, in the present preliminary study, we have found increased levels of GM-CSF, a cytokine that may be produced both by Th-1 and Th-2 T-cells, in the lungs of chronic bronchitic patients with an exacerbation. Thus, the increased GM-CSF levels in the lower respiratory tract of asthmatics (particularly during an asthmatic reaction) and in the lower respiratory tract of patients with chronic bronchitis (particularly during an exacerbation), associated with the bronchial eosinophilia during exacerbations of chronic bronchitis, seem to suggest that both of these "acute stages" of the two different diseases might be supported by similar pattern of cell-cytokine networks.

Further studies, including larger number of patients, are needed to characterize the inflammation in the lower respiratory tract during an exacerbation of chronic bronchitis.

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