

Contribution of bronchial biopsies in the evaluation of pathogenesis and progression of COPD

F. Magno¹, A. Di Stefano²

ABSTRACT: *Contribution of bronchial biopsies in the evaluation of pathogenesis and progression of COPD.*
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This review summarizes and discusses the lung pathology of COPD patients emphasising on inflammatory cell phenotypes and mechanisms which prevail in different clinical conditions.

In bronchial biopsies a series of events takes place during the progression of the disease from mild to severe. T-lymphocytes, particularly CD8⁺ cells and macrophages are the prevalent inflammatory cells in the lungs of healthy smokers and patients with mild/moderate COPD. This T-cell activation seems to be sustained by CD4⁺, CD8⁺ cells and macrophages expressing transcription factors and Tc1 cytokines such as NF-kB, STAT4 and IFN γ .

In contrast, severe disease is characterized by lymphocytes producing greater amounts of TGF- β 1 and by an increase of nitrotyrosine immunoreactivity and activated neutrophils, macrophages and MPO⁺ cells. However, the mechanisms involved in neutrophilic migration and adhesion are currently under investigation. Recent data has shown that in severe COPD there is an impaired neutrophil capability to respond to chemotactic stimuli, as well as an increased collagen adhesion of neutrophils due to the up-regulation of CD44 and CD11b receptors. This data together, may account for the increased neutrophilia observed in the severe disease states of COPD. In this context, insights obtained from the tissutal analysis of bronchial biopsies represent an irreplaceable route to further progresses in to the pathogenesis of this disease.
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Keywords: COPD, inflammatory cells, bronchial biopsies.

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Introduction

Chronic obstructive pulmonary disease (COPD) can be defined as chronic irreversible and progressive airflow limitation associated with an abnormal inflammatory response of the lung and can be characterised by a slowly progressive and irreversible deterioration in lung function [1, 2]. COPD is characterised by the destruction of the lung parenchyma and enlargement of airspaces, typical of pulmonary emphysema, inflammation and obstruction of the peripheral airways, and inflammation of the central airways. Pathological studies show that inflammation in COPD occurs in the central and peripheral airways (bronchioles) and lung parenchyma. Cigarette smoking is the major risk factor for the development of COPD and cigarette smokers constitute over 90% of COPD patients in developed countries [1, 2]. However, even if smokers with COPD stop smoking, a continuous cycle of inflammation can lead to a continued decline in lung function underlying the role of inflammatory cells for development and maintenance of this disease [3]. In the last decade many studies performed on bronchial biopsies have focused on the inflammatory process, cellular changes and structural alterations developing in

stable mild/moderate COPD [4, 5] and in the severe disease state [6, 7]. These studies on the pathology of the lung in this disease have contributed significantly to the present knowledge on the structural alterations associated with airflow limitation in different clinical conditions. Previous papers have extensively reviewed the structural alterations occurring in the lung of patients with COPD [8, 9]. However, these have not been focused on changes occurring with different degrees of disease severity [8, 10, 11]. In this review article, we will focus our attention on studies that have analysed bronchial biopsies from COPD patients suffering from mild to severe forms, giving attention on the causes of progression of this disease. Recent data focused on possible mechanisms inducing the specific cellular pattern in the severe disease is also discussed.

Mild/moderate COPD

INFLAMMATORY CELLS

Analysis of inflammatory cell infiltration in bronchial biopsies of patients with mild/moderate COPD shows an increased inflammatory cell infiltration in comparison with control non-smokers [4, 11]. All authors agree on the observation that T-lymphocytes, mainly CD8⁺ cells, and macrophages

(CD68+ cells) prevail in the bronchial mucosa of these patients [4, 11]. When mild/moderate COPD patients are compared with control smokers, matched by age and smoking habit, no significant differences were reported in the numbers of CD3+ and CD8+ cells in the submucosa [12, 13]. Smokers with normal lung function also showed, though to a lesser extent, increased numbers of CD3+ and CD8+ cells compared with control non-smokers [7, 13]. This data suggests that the T-lymphocyte increase may be an effect of smoking which occurs in a wide range of smokers [7, 11]. This raises doubts as to the validity of the O'Shaughnessy's speculation that increases in CD8+ cells are restricted to a small subgroup of smokers who develop airflow limitation [14, 15]. An increased presence of CD8+ cells had also been previously documented in the bronchoalveolar lavage (BAL) and blood of smokers [16, 17]. However, the CD3+ and particularly the CD8+ functional activity remains under investigation. One study reported that CD8+ lymphocyte populations of patients with COPD demonstrate a higher degree of activation compared with control subjects and this abnormality positively correlated with disease severity. Recently, it was found that the volume of B-cells in bronchial biopsies of large and small airways is higher in patients with mild/moderate COPD in comparison with controls without airflow limitation but lower than in severe COPD; however any relationship was found between the number of these cells and clinical characteristics within the COPD diseased group [18]. Furthermore, increased neutrophil presence has been reported in sputum [19, 20] and BAL fluid [21, 22] of mild/moderate COPD patients in comparison with controls.

MOLECULAR CASCADE

The observation of ELAM-1 overexpression in endothelial cells [12] and of IL-8 in primary cultured bronchial epithelial cells [23] provide a possible mechanism involved in the neutrophil recruitment in COPD. In fact adhesion molecule ELAM-1 in endothelium, and ICAM-1 in bronchial epithelium have been reported to be increased in mild/moderate disease in comparison with both control non-smokers and current smokers [12, 23, 24]. Overexpression of ICAM-1 in epithelium may play a role in the T-cell epithelial adhesion [12] and T-cell mediated response to viral infections [12, 25]. The chemokine receptor CCR5, preferentially expressed by Th1/Tc1 cells producing IFN γ , was reported to be increased in mild/moderate disease in comparison with control smokers [7], suggesting that, at variance with asthma, a prevalent Th1/Tc1 immunosurveillance develops in mild/moderate COPD patients in stable conditions. We [26] recently reported increased Stat-4 (Signal Transducer and Activators of Transcription) nuclear expression in bronchial epithelium and submucosa of mild/moderate COPD patients in comparison with both control smokers and non-smokers. Stat-4 is critical for the differentiation of Th1/Tc1 cells [27, 28] and its nuclear expression is correlated significantly with the num-

ber of IFN- γ +, CD3+ and CD4+ cells but not with CD8+ cells in the submucosa of smokers.

The epithelial and nuclear expression of the transcription factor NF- κ B (p65 protein) was increased in mild/moderate COPD and, to a lesser extent, in control smokers in comparison with control non-smokers [13]. Nuclear expression of NF- κ B can modulate the transcription of a number of pro-inflammatory cytokines such as IL-1, IL-6, IL-8, MCP-1, TNF α and ICAM-1 [13] (table 1).

Severe COPD

INFLAMMATORY CELLS

In severe COPD a decreased number of T-lymphocytes (CD3+ cells) and of CD3+ cells coexpressing the CCR5 receptor (CCR5+CD3+ cells) were reported in comparison with both mild/moderate COPD patients and control smokers (7). In contrast, the number of neutrophils and macrophages (CD68+) in comparison with control non smokers was reported to be increased [6].

In the submucosa an increased number of myeloperoxidase+ (MPO) and nitrotyrosine+ (NT) cells was discovered, in comparison with mild/moderate COPD and controls [29]; the number of MPO+ cells was significantly correlated with the number of neutrophils in the bronchial submucosa [30]. iNOS+ and eNOS+ but not XO+ cells were significantly increased in smokers with COPD or normal lung function compared with non smokers [30]. These data show that a nitrosative stress plays a role in severe disease.

MOLECULAR CASCADE

Neutrophilic airway inflammation is considered to be a major factor in the pathogenesis of COPD. Neutrophils are a source of proteolytic enzymes thought to contribute to the destruction of the lung parenchyma and of oxidants who acts also on bronchial cells. In the bronchial epithelium of severe COPD patients it was reported that there was an increased expression of macrophage inflammatory protein-1 (MIP-1 α), a chemokine inducing migration and activation of mononuclear cells and granulocytes in comparison with subjects with mild/moderate disease and control smokers [6]. Recent studies investigating the mechanisms responsible for neutrophil accumulation, demonstrated that neither the increased chemotactic responsiveness of neutrophils [31], nor the prolonged survival of these cells in sputum [32] of COPD patients could explain the increased numbers of airway neutrophils in this disease. Our observation of an increased percentage of neutrophils coexpressing the CD44 (CD44+Neu+) and CD11b (CD11b+Neu+) antigens in severe COPD compared to control smokers, let us to speculate that increased presence of chemotactic factors (NAP-2, RANTES) for neutrophils together with an increased neutrophil adhesiveness to the submucosal collagen components (hyaluronic acid) and adhesion molecules, mediated by the overexpression of CD44 and CD11b, could play a role in sustaining neutrophilia in patients with severe COPD [32-33] (fig. 2). No significant differences were observed

on the expression of CXCL8(IL-8), CXCL1(GRO-alpha) and CXCL5(ENA 78) in the submucosa from severe COPD patients compared to control non smokers [33]. However in all smokers a positive correlation was observed between IL-8 epithelial expression and the number of neutrophils in the submucosa [24] (table 1).

The analysis of bronchial biopsies has yielded much information on the role of inflammatory cells in the pathogenesis of this disease. T-lymphocytes. In particular CD8+ cells and macrophages, are the predominant inflammatory cells in the bronchial mucosa of all smokers with and without mild air-flow limitation, while neutrophils and macrophages

Table 1. - Variations of markers of inflammation in the bronchial submucosa and *epithelium* of control smokers, non smokers and COPD patients with different degrees of disease severity

| | |
|--|---|
| SEVERE COPD | CONTROL SMOKERS |
| ↑ MPO NT NAP-2 RANTES MIP-1α | ↑ TGF-β NF-κB (p65) NF-κB (p65) MPO NT → ELAM-1 IFN-γ STAT-4 |
| MILD/MODERATE COPD | CONTROL NON SMOKERS |
| ↑ ELAM-1 TGF-β IFN-γ STAT-4 NAP-2 NF-κB (p65) ICAM-1 IL-8 → MPO NT | → ELAM-1 TGF-β IFN-γ STAT-4 NF-κB (p65) MPO NT |

↑ : significantly increased values in comparison with that indicated by →
 → : basal values or values non-significantly changed

ELAM-1, endothelial adhesion molecule-1; NF-κB (p65), nuclear factor-kappa B; STAT-4, signal transducer and activators of transcription; MPO, myeloperoxidase; NT, nitrotyrosine; TGF-β, transforming growth factor-beta; ICAM-1, intercellular adhesion molecule-1; MIP-1α, macrophage inflammatory protein-1.

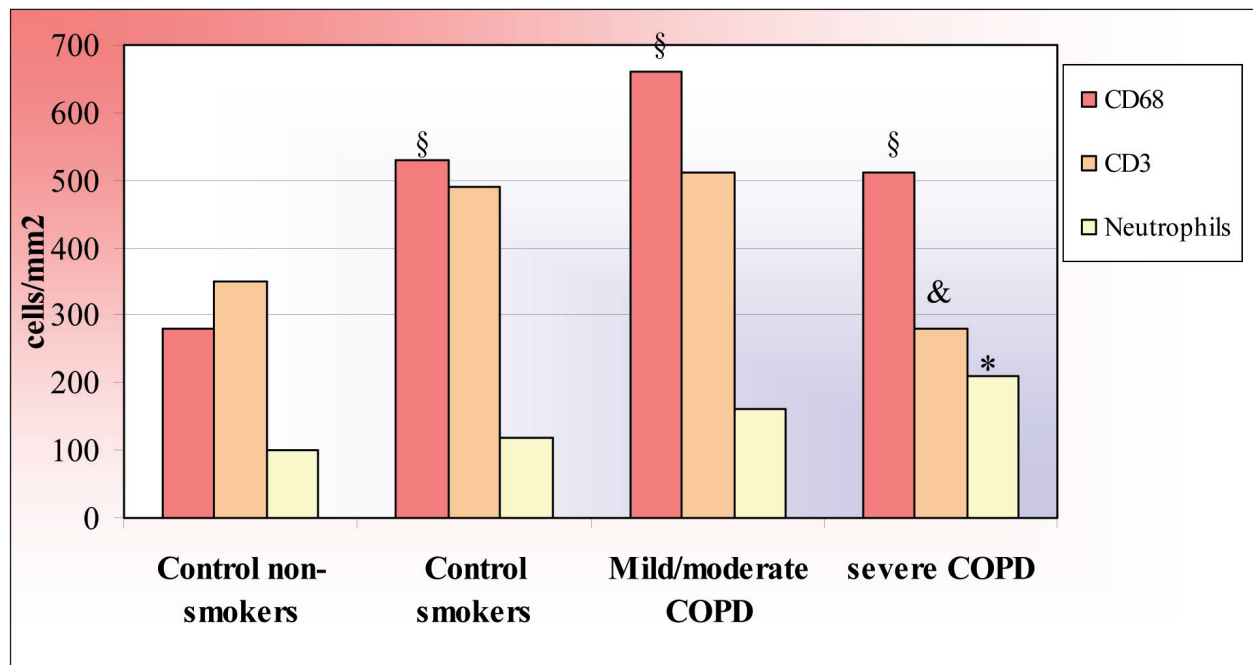


Fig. 1. - Schematic representation of the cellular variations in the bronchial biopsy submucosa of control non smokers, control smokers and COPD patients with mild/moderate and severe disease. Graph based on reference [6, 7].
 * significantly different from control non smokers, control smokers and mild/moderate COPD;
 § significantly different from control non smokers;
 & significantly different from mild/moderate COPD and control smokers.

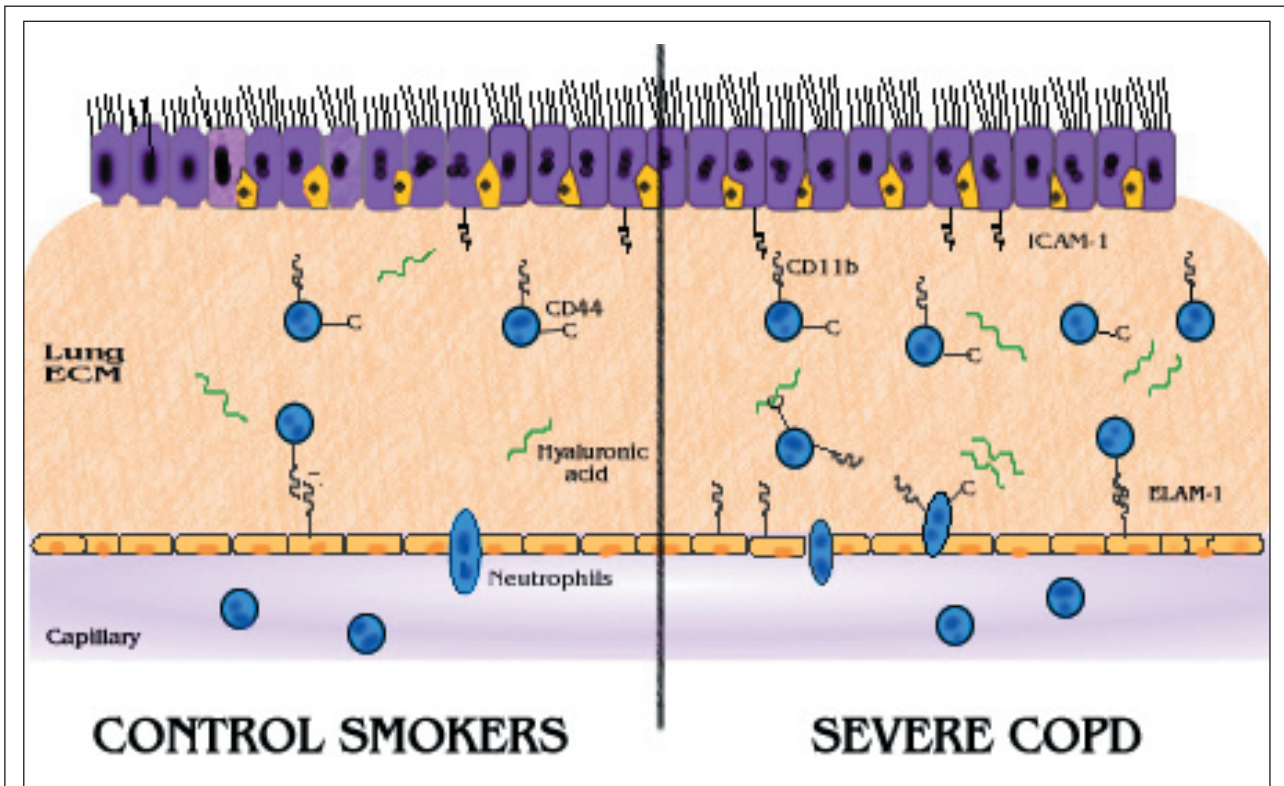


Fig. 2. - Schematic representation of the bronchial submucosa from a control smoker and a patient with severe COPD. In the bronchial mucosa from severe COPD there is an increased percentage of neutrophils coexpressing the CD44 (CD44+Neu+) and CD11b (CD11b+Neu+) antigens as well as an increased expression of CD44 and CD11b ligands, ICAM-1, ELAM-1 and hyaluronic acid. The interaction between ligands in the submucosal collagen and their receptors in the neutrophil surface may account for the increased presence of neutrophils in severe COPD patients.

predominate in the most severe forms of COPD (fig. 1). The molecular cascade and the regulatory mechanisms involved in the regulation of T-lymphocytes and neutrophils predominance in mild and severe disease, respectively, need to be studied. Up-regulation of pro-inflammatory transcription factors NF- κ B and STAT-4 in mild COPD, and activated epithelial and endothelial cells in the more severe disease may contribute to this differential prevalence of infiltrating cells. To this purpose bronchial epithelial and endothelial cell alterations need to be studied in more detail. In bronchial biopsies from COPD patients, the overexpression of IL-8, NAP-2 and RANTES and the increased percentages of neutrophils coexpressing adhesive receptors such as CD44 and CD11b may play a role in sustaining neutrophilia in these clinical conditions. Impairment of neutrophilic response to chemotactic stimuli may also contribute to this increased neutrophilia.

While the inflammatory cell picture of the severe to mild disease is becoming clearer, the analysis of the molecular mechanisms underlying COPD development has only just begun. At variance with inflammatory cells coming from BAL and sputum obtained from different airway compartments, bronchial biopsy analysis allow us to investigate the molecular characteristics of resident and prevalent inflammatory cells acting directly in the bronchial tissue. In this context, insights obtained from the analysis of bronchial biopsies represent an irreplaceable route to further progresses in to the pathogenesis of this disease.

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