

REVIEW

Cellular and molecular mechanisms in chronic obstructive pulmonary disease: an overview

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Summary

In the last decade, the analysis of bronchial biopsies and lung parenchyma obtained from chronic obstructive pulmonary disease (COPD) patients compared with those from smokers with normal lung function and non-smokers has provided new insights on the role of the different inflammatory and structural cells, their signalling pathways and mediators, contributing to a better knowledge of the pathogenesis of COPD. This review summarizes and discusses the lung pathology of COPD patients with emphasis on inflammatory cell phenotypes that predominate in different clinical conditions. In bronchial biopsies, a cascade of events takes place during progression from mild-to-severe disease. T lymphocytes, particularly CD8⁺ cells and macrophages are the prevalent inflammatory cells in the lung of healthy smokers and patients with mild COPD, while total and activated neutrophils predominate in severe COPD. The number of CD4⁺, CD8⁺ cells and macrophages expressing nuclear factor-kappa B (NF-κB), STAT-4 and IFN-γ proteins as well as endothelial adhesion molecule-1 in endothelium is increased in mild/moderate disease. In contrast, activated neutrophils (MPO⁺ cells) and increased nitrotyrosine immunoreactivity develops in severe COPD. In bronchial biopsies obtained during COPD exacerbations, some studies have shown an increased T cell and granulocyte infiltration. Regular treatment with high doses of inhaled glucocorticoids does not significantly change the number of inflammatory cells in bronchial biopsies from patients with moderate COPD. The profile in lung parenchyma is similar to bronchial biopsies. 'Healthy' smokers and mild/moderate diseased patients show increased T lymphocyte infiltration in the peripheral airways. Pulmonary emphysema is associated with a general increase of inflammatory cells in the alveolar septa. The molecular mechanisms driving the lymphocyte and neutrophilic prevalence in mild and severe disease, respectively, needs to be extensively studied. Up-regulation of pro-inflammatory transcription factors NF-κB and STAT-4 in mild, activated epithelial and endothelial cells in the more severe disease may contribute to this differential prevalence of infiltrating cells.

Introduction

Chronic obstructive pulmonary disease (COPD) can be defined as chronic irreversible airflow limitation confirmed by spirometry [1–3] and is characterized by a slowly progressive and irreversible deterioration in lung function [1–3]. Cigarette smoking is the major risk factor for the development of COPD and cigarette smokers constitute over 90% of COPD patients in developed countries [1–3]. The pathological hallmarks of COPD are destruction of the lung parenchyma, which characterizes pulmonary emphysema, inflammation of the peripheral airways, which characterizes respiratory bronchiolitis, and inflammation of the central airways. Pathological studies show that inflammation in COPD occurs in the central and peripheral airways (bronchioles) and lung parenchyma.

We will focus our attention on studies that have analysed bronchial biopsies or specimens from bronchial rings and peripheral lung tissue obtained during lung resection for a peripheral carcinoma (see Tables 1–3). Data obtained on lung parenchyma by lung volume reduction surgery (LVRS) performed in patients with emphysema have also been reported because they represent almost the only source of tissue specimens from severely diseased patients with pulmonary emphysema.

It should be noted that in the last 15 years, the definition of COPD in the national and international guidelines has changed many times [1–4]. This could explain some discrepancies in results observed in the studies reviewed here (see Tables 1–3).

Recent studies performed on bronchial biopsies have focused on characterizing the inflammatory process and structural alterations developing in stable mild/moderate COPD [5–12] and in the mild forms of COPD during exacerbations [13, 14]. The severe disease state has also been recently investigated [10, 11]. Bronchial biopsy changes after corticosteroid treatment of stable moderate COPD have also been evaluated

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Table 1. Variations of inflammatory cells and markers of inflammation in the bronchial submucosa of COPD patients, control smokers and non-smokers

	CD45	CD3	Neutr	EOS	Mast	CD25	CD68	ELAM-1	CD8	TGF- β	NK	CD4	CCR5-CD3	NF- κ B (p65)	IFN- γ	STAT-4	MPO	NT
Severe COPD	—	↓ ⁷	↑ ⁶	→ ⁶	→ ⁶	—	↑ ⁶	—	↓ ⁷	—	↑ ⁶	→ ⁷	↓ ⁷	—	—	—	↑ ¹⁰	↑ ¹⁰
Mild/moderate COPD	↑ ^{2,4}	↑ ^{1,2,4,8}	→ ^{2,4}	→ ^{2,4,6}	→ ^{2,4,6}	↑ ²	↑ ^{2,4}	↑ ³	↑ ^{4,8}	↑ ⁵	→ ⁶	→ ^{2,4,7,8}	→ ⁷	↑ ⁸	↑ ⁹	↑ ⁹	→ ¹⁰	→ ¹⁰
Control smokers	→ ⁴	→ ^{4,7}	→ ^{4,6}	→ ^{4,6}	→ ^{4,6}	→ ⁴	→ ^{4,6}	→ ³	↑ ⁸	↑ ⁵	→ ⁶	→ ^{4,7,8}	→ ⁷	↑ ⁸	→ ⁹	→ ⁹	→ ¹⁰	→ ¹⁰
Control non-smokers	→ ^{2,4}	→ ^{1,2,4,8}	→ ^{2,4}	→ ^{2,4}	→ ^{2,4}	→ ^{2,4}	→ ^{2,4}	→ ³	→ ^{2,4,8}	→ ⁵	—	→ ^{2,4,8}	—	→ ⁸	→ ⁹	→ ⁹	→ ¹⁰	→ ¹⁰

1, from reference [5]; 2, from reference [6]; 3, from reference [9]; 4, from reference [22]; 5, from reference [12]; 6, from reference [10]; 7, from reference [11]; 8, from reference [21]; 9, from reference [30]; 10, from reference [37]

↑, significantly increased values in comparison with that indicated by →; →, basal values or values non-significantly changed; ↓, significantly decreased values in comparison with that indicated by →.

Numbers close to the arrows indicate references.

ELAM-1, endothelial adhesion molecule-1; NK, natural killer; NF- κ B, nuclear factor-kappa B; STAT-4, signal transducer and activators of transcription; MPO, myeloperoxidase; NT, nitrotyrosine; COPD, chronic obstructive pulmonary disease.

Table 2. Variations of inflammatory cells and markers of inflammation in the central and peripheral airways obtained from surgical specimens of COPD patients, chronic bronchitics with normal lung function and control smokers

	Central airways							Peripheral airways (< 3mm Ø)					
	Neutr	CD68	CD4	CD8	IL-4	IL-5	Total inflam.	Neutr	CD68	CD4	CD8	TGF- β 1	Total inflam.
Mild/moderate* COPD	→ ²	→ ²	→ ²	→ ²	↓ ¹	→ ¹	↓ ¹	→ ^{4,5,7,9}	↑ ^{6,9}	→ ^{4,5,6,7,9}	↑ ⁴	↑ ⁶	↑ ⁴
							→ ²		→ ^{4,5,7}		→ ^{5,6,7,9}	→ ¹⁰	→ ^{5,6,7,9}
Chronic* bronchitis with normal FEV ₁	—	—	—	—	↑ ¹	→ ¹	↑ ^{1,3}	—	—	—	—	—	↑ ⁸
Control* smokers	→ ²	→ ²	→ ²	→ ²	→ ¹	→ ¹	→ ^{1,3}	→ ^{4,5,7,9}	→ ^{4,5,6,7,9}	→ ^{4,5,6,7,9}	→ ^{4,5,6,7,9}	→ ^{6,10}	→ ^{4,5,6,7,8,9}

1, from reference [26]; 2, from reference [56]; 3, from reference [55]; 4, from reference [60]; 5, from reference [61]; 6, from reference [63]; 7, from reference [62]; 8, from reference [57]; 9, from reference [67]; 10, from reference [64]

↑, significantly increased values in comparison with that indicated by →; →, basal values or values non-significantly changed; ↓, significantly decreased values in comparison with that indicated by →.

Numbers close to the arrows indicate references.

*Specimens obtained from resected lungs for localized tumours.

FEV₁, forced expiratory volume in 1 s; COPD, chronic obstructive pulmonary disease.

[15]. Structural alterations in the peripheral airways and parenchyma have been investigated mostly in mild forms of COPD and, more rarely, in severe disease. These studies on the pathology of the lung in this disease have contributed significantly to the present knowledge on the structural alterations associated to airflow limitation in different clinical conditions. Previous papers have extensively reviewed the structural alterations occurring in the lung of patients with COPD [16–18]. However, these have not focused on changes occurring with different degrees of disease severity [16–20]. In the last few years, studies on this topic have made important

contributions that have extended the previous notions on the inflammatory cell prevalence in the large and small airways and parenchyma. Furthermore, important preliminary findings are, at present, available on the molecular mechanisms involved in the development of COPD and pulmonary emphysema. Planning of well-focused future studies requires a clear preliminary picture on the inflammatory cells and mediators that predominate in COPD at different clinical stages and after pharmacologic treatment. We review here the contributions made over the last $\approx$ 15 years, based on the analysis of bronchial biopsies of COPD patients in different

Table 3. Variations of inflammatory cells in the lung parenchyma obtained from surgical specimens of mild/moderate COPD patients, smokers with normal lung function and control non-smokers

	Neutr	CD68	CD3	CD4	CD8	Eosin	TGF- β 1	Total inflam.
Mild/moderate* COPD	→ ^{2,3}	→ ²	↑ ³	→ ^{2,3}	↑ ^{2,3}	→ ²	↑ ⁴	→ ³
Smokers* with normal FEV₁	→ ^{1,2,3}	↑ ¹ → ²	↑ ¹ → ³	→ ^{2,3}	→ ^{2,3}	→ ²	→ ⁴	→ ³
Control* non-smokers	↑ ¹ → ^{2,3}	→ ^{1,2}	→ ^{1,3}	→ ^{2,3}	→ ^{2,3}	→ ²	—	→ ³

1, from reference [68]; 2, from reference [70]; 3, from reference [71]; 4, from reference [72]

↑, significantly increased values in comparison with that indicated by →; →, basal values or values non-significantly changed; ↓, significantly decreased values in comparison with that indicated by →.

Numbers close to the arrows indicate references.

*Specimens obtained from resected lungs for localized tumours.

FEV₁, forced expiratory volume in 1 s; COPD, chronic obstructive pulmonary disease.

clinical stages and after steroid treatment and of specimens from resected lung in COPD patients with stable disease.

Bronchial biopsies

Bronchial biopsies in stable chronic obstructive pulmonary disease

Mild/moderate chronic obstructive pulmonary disease vs. control smokers and non-smokers 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 [5–9]. All authors agree on the observation that T lymphocytes, mainly CD8⁺ cells, and macrophages (CD68⁺ cells) predominate in the bronchial mucosa of these patients [5–9]. When mild/moderate COPD patients are compared with control smokers, matched for age and smoking habit, we reported no significant differences in the numbers of CD3⁺ and CD8⁺ cells in the submucosa [21]. Smokers with normal lung function also showed, though to a lesser extent, increased numbers of CD3⁺ and CD8⁺ cells compared with control non-smokers [11, 21] (Table 1). These data suggest that the T lymphocyte increases may be an effect of smoking that occurs in a wide range of smokers. This rises 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 [22, 23]. An increased presence of CD8⁺ cells had also been previously documented in the bronchoalveolar lavage (BAL) and blood of smokers [24, 25]. However, the CD3⁺ and particularly the CD8⁺ functional activity remains under investigation. Zhu et al. [26] reported that in central airways from surgical specimens of smokers with chronic bronchitis, 15% of CD8⁺ cells coexpress TNF- α protein. Perforin and granzyme as well as Fas-L-mediated

cytotoxicity, potentially expressed by CD8⁺ cells, have not been studied in bronchial biopsies or surgical specimens of COPD patients. Liu et al. [27] reported a TNF- α -dependent as well as perforin and Fas-L-independent cytolytic activity of mice CD8⁺ cells against cultured alveolar epithelial cells [27]. If these *in vitro* observations are confirmed by further *in vivo* studies, cytotoxicity of CD8⁺ cells in the bronchial mucosa of smokers could be restricted to a limited percentage of CD8⁺ cells (15% in Zhu's study) and could depend on the production and release of TNF- α protein. The question, however, of the role and functions of increased CD8⁺ cells populating the bronchial mucosa of smokers remains, at least in part, open. The results of ongoing trials of anti-TNF- α therapy in COPD may help clarify the role of this cytokine.

The chemokine receptor CCR5, preferentially expressed by T-helper type 1 (Th1)/T-cytotoxic (Tc1) cells producing IFN- γ , was reported to be increased in mild/moderate disease in comparison with control smokers [11], suggesting that, at variance with asthma, a prevalent Th1/Tc1 immunosurveillance develops in mild/moderate COPD patients in stable conditions. The expression of the transcription factor signal transducer and activators of transcription (STAT)-4 is critical for the differentiation of Th1/Tc1 cells [28, 29]. We recently reported increased STAT-4 nuclear expression in bronchial epithelium and submucosa of mild/moderate COPD patients in comparison with both control smokers and non-smokers [30]. STAT-4 nuclear expression correlated significantly with the number of IFN- γ ⁺, CD3⁺ and CD4⁺ cells but not with CD8⁺ cells in the submucosa of smokers: 50% of CD4⁺, one-third of CD8⁺ and one-third of CD68⁺ cells in the bronchial submucosa coexpressed the STAT-4 protein. These data on the expression of the transcription factor STAT-4 in COPD patients reinforce the notion of a prevalent Th1/Tc1 immunological response in mild/moderate disease.

The demonstration that the production of IL-4 in the glands of chronic bronchitis is independent of CD8⁺ lymphocytes, as reported by Zhu et al. [26], again suggests that CD4⁺ lymphocytes could be mainly involved in the Th2 cytokine response associated with hypersecretion of mucous glands in smokers. In the light of these results, the Th1 or Th2 response involving CD4⁺ cells, may be functionally important in COPD, even though their relative number has not been reported as increased in comparison with control subjects.

The epithelial and nuclear expression of the transcription factor nuclear factor-kappa B (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 [21]. We reported that approximately one-third of CD4⁺ and CD8⁺ T lymphocytes coexpressed the p65 NF- κ B subunit in the bronchial biopsies of COPD patients. 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, monocyte chemoattractant protein-1 (MCP-1), TNF- α and intercellular adhesion molecule-1 (ICAM-1) [21]. Enhanced nuclear expression of NF- κ B demonstrates the activation of a key molecular mechanism involved in the ongoing inflammatory process in the airways of these subjects.

Endothelial adhesion molecule (ELAM-1) in endothelium, ICAM-1 and IL-8 in bronchial epithelium have been reported to be increased in mild/moderate disease in comparison with both control non-smokers and current smokers [9, 31]. Since neutrophils accumulate in the sputum [32, 33] and BAL fluid [34, 35] of COPD patients, the observation of ELAM-1 overexpression in endothelial cells [9] and of IL-8 in primary cultured bronchial epithelial cells [31] provide a possible mechanism involved in the neutrophil recruitment in COPD. Overexpression of ICAM-1 in epithelium may play a role in the T cell epithelial adhesion [9] and T cell-mediated response to viral infections [9, 36]. Thickness of the subepithelial basement membrane was reported to be similar [5, 6] or slightly increased [12] in COPD patients compared with control smokers and non-smokers. The selection criteria of COPD patients and the possible inclusion of few asthmatic patients may have influenced the results obtained in these studies [7, 12]. The prevalent view, however, is that this lesion is a characteristic feature of asthmatic bronchi but is not present in COPD patients [5, 6, 17].

Severe chronic obstructive pulmonary disease vs. mild/moderate disease and control groups

We have reported increased expression of macrophage inflammatory protein-1 (MIP-1 α), a chemokine inducing migration and activation of mononuclear cells and granulocytes, in the bronchial epithelium of severe COPD patients in comparison with subjects with mild/moderate disease and control smokers [10]. We also found increased myeloperoxidase (MPO) immunoreactivity in comparison with mild/moderate disease and both control groups [37]. In the submucosa, we reported a further increase of neutrophils and macrophages (CD68⁺) in comparison with control smokers [10] and decreased numbers of T lymphocytes (CD3⁺ cells) and of CD3⁺ cells coexpressing the CCR5 receptor (CCR5⁺ CD3⁺ cells) in comparison with both mild/moderate COPD patients and control smokers [11] (Figs 1 and 2, Table 4).

These data show a shift of the cellular types involved in severe disease, with a prevalence of cells possessing phagocytic and proteolytic activity in the bronchial tissue (Fig. 2). In contrast, the T cell-mediated immunoresponse could be impaired or modified in COPD patients with severe disease [11], in concomitance with the decrease in number of T cells in these clinical conditions. This speculation is also in line with *in vitro* studies showing that neutrophil-derived products can inhibit lymphocyte antigen expression of CD2, CD3, CD4 and CD8 [38] and that CCR5 receptor expression is negatively correlated with numbers of neutrophils in the BAL fluid of patients with sarcoidosis and chronic bronchitis [39].

We have also shown increased MPO⁺ and nitrotyrosine⁺ (NT) cells in the submucosa of severely diseased patients in comparison with mild/moderate COPD, control smokers and non-smokers [37]. Since NT formation is related to peroxynitrite activity, which causes tissue damage, we suggested that interaction between MPO and nitrites (NO₂⁻), a mechanism already reported as effective in different inflammatory conditions [40], may cause tissue damage, small pro-inflammatory peptides release and increased adhesion and activation of tissue neutrophils and macrophages. Since IL-8 induces release of MPO from neutrophils [41], IL-8 secreted by bronchial epithelium, neutrophil themselves and macrophages producing IL-8 [42], may further sustain this inflammatory process. One could argue that fibrotic events and remodelling may take place in the central airways at this stage of the disease in association with the changes in the cellular types and in their state of activation in the bronchial mucosa. However, the molecular characteristics of the extracellular matrix and its potential role in the recruitment, adhesion and activation of neutrophils and macrophages [43], as well as the activation state of these cells, need to be more extensively studied in bronchial biopsies of patients with moderate/severe disease. At variance with asthma, the functional activity of bronchial epithelium in COPD patients is poorly studied (Table 4 and Fig. 4).

One limitation of the above-mentioned studies, particularly those in patients with severe disease, is the glucocorticoid treatment frequently assigned to such patients. It is not known whether a wash-out period of 1 month before bronchoscopy and biopsy collecting, adopted in most protocol studies involving bronchial biopsy collection, is really sufficient to avoid the influence of treatment on the inflammatory cell infiltration. Even though steroids have been recently reported to have little influence on the inflammatory picture seen in bronchial biopsies of glucocorticoids-treated COPD patients [15], we cannot exclude a possible effect of prolonged treatments with glucocorticoids when bronchial biopsies from moderate/severe diseased patients are analysed.

Bronchial biopsies in stable chronic obstructive pulmonary disease after treatment with glucocorticoids

Few data are available in the literature regarding inflammatory changes after treatment with glucocorticoids in the bronchial biopsies of COPD patients. Hattotuwa et al. [15] compared bronchial biopsies from a group of moderate COPD patients after 3 months of inhaled glucocorticoids with those from a placebo-treated group. The authors observed a significant decrease in numbers of exacerbations and symptoms

score together with a decreased number of mast cells in the bronchial mucosa of glucocorticoid-treated group. However, the number of total leucocytes (CD45⁺ cells), eosinophils, neutrophils, CD4⁺ and CD8⁺ cells did not differ in the two

groups. The number of CD4⁺ cells was increased in the placebo compared with glucocorticoid-treated group [15]. These data suggest that in stable COPD, glucocorticoid treatment positively influences symptoms and exacerbations, but has little influence on the inflammatory process involving the airways. Data obtained from sputum analysis [44, 45] and BAL [46, 47] are in agreement with the few observations reported for bronchial biopsies. Recently, a possible mechanism that underlie, at least in part, glucocorticoid insensitivity in inflammatory cells of smokers with COPD has been proposed. Histone deacetylase (HDAC)-2 expression, which is essential for the suppression of many inflammatory genes by glucocorticoids, is decreased in the bronchial biopsies and in the macrophages from BAL of cigarette smokers [48]. This results in reduced glucocorticoid responsiveness and increased histone acetylation, which in turn leads to a subsequent increase in inflammatory cytokine expression. This observation suggests a possible mechanism involved in the decreased efficacy of glucocorticoids in subjects exposed to cigarette smoke.

Bronchial biopsies during exacerbations of chronic obstructive pulmonary disease

Patients with mild/moderate COPD during an exacerbation of the disease show an increased number of eosinophils in their bronchial mucosa and increased mRNA for RANTES, neutrophils, T lymphocytes, very late antigen-1 and TNF- α in comparison with stable COPD patients [13, 14]. Increased eotaxin-1 and CCR3 chemokine receptor expression has also been reported in such patients in comparison with control non-smokers [49]. ELAM-1 and ICAM-1 proteins, as well as mRNA for IL-4 and IL-5 were not changed during COPD exacerbations compared with stable disease [13, 14]. Severe exacerbations of COPD were associated with increased neutrophilia and up-regulation of epithelial mRNA for CXCL-5 (ENA-78), CXCL-8 (IL-8), CXCR-1 and CXCR-2 in comparison with stable disease [50]. These data show that total inflammation, involving different inflammatory cells, is significantly increased during COPD exacerbations. However, the eosinophilia observed during exacerbations in mild/moderate COPD, differs from that reported in asthma. In fact, during COPD exacerbations, the presence of eosinophils is transitory and frequently confined to capillary vessels (Fig. 3). In stable disease, eosinophils have been occasionally described, but it is generally accepted that eosinophils are not a cellular type generally populating the bronchial mucosa of patients with mild/moderate COPD, as occurs in mild/moderate asthma. Furthermore, apart from increased RANTES [14], other mechanisms involved in the eosinophil

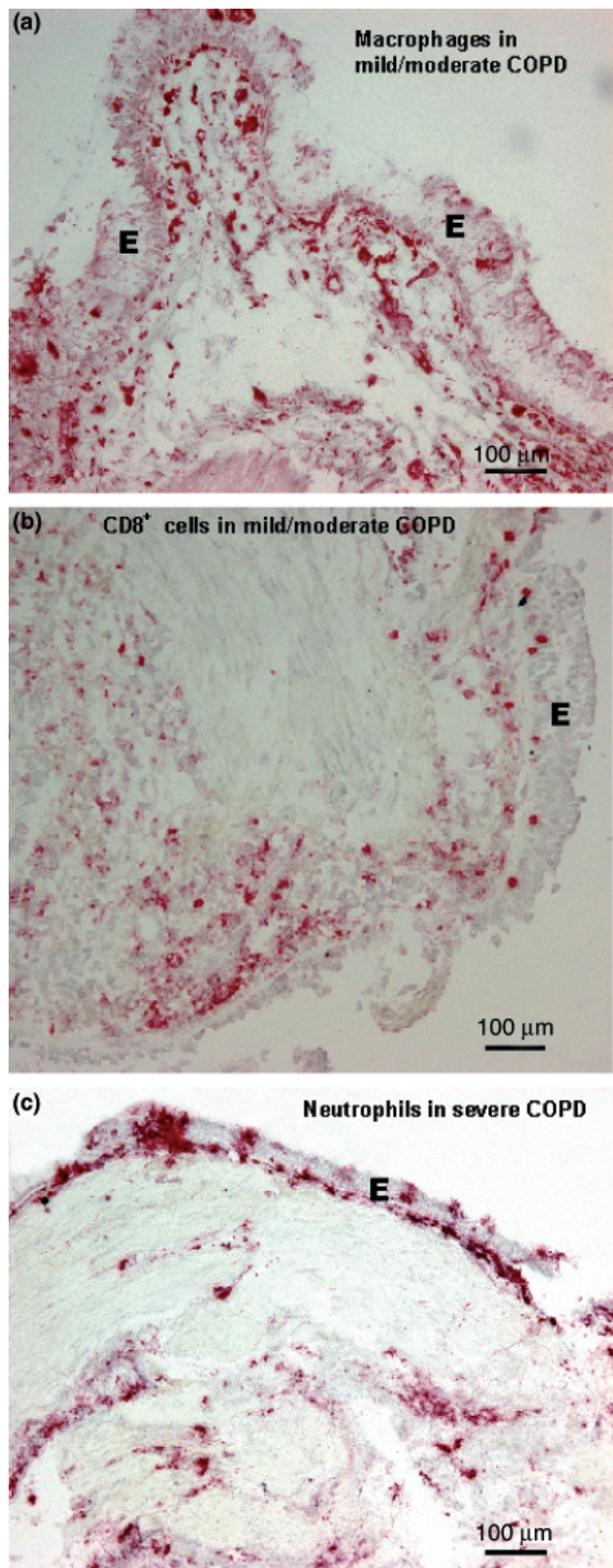


Fig. 1. Photomicrographs showing bronchial biopsies of patients with mild/moderate chronic obstructive pulmonary disease (COPD) immunostained for identification of (a) macrophages (CD68⁺ cells) (red stain), (b) CD8⁺ lymphocytes (red stain) and (c) of a patient with severe COPD immunostained for identification of neutrophils (red stain). Results are representative of the CD68⁺, CD8⁺ cells and neutrophils in mild/moderate and severe disease, respectively. Infiltration of CD68⁺ cells is frequently extended to the whole submucosal area of the bronchial biopsies. Neutrophils and CD8⁺ cells are more frequently localized in the more superficial layer of the bronchial mucosa. Macrophages (CD68⁺ cells) and CD8⁺ lymphocytes predominate in mild/moderate COPD; neutrophils and macrophages predominate in severe disease. Original magnification: $\times 10$. E, epithelium.

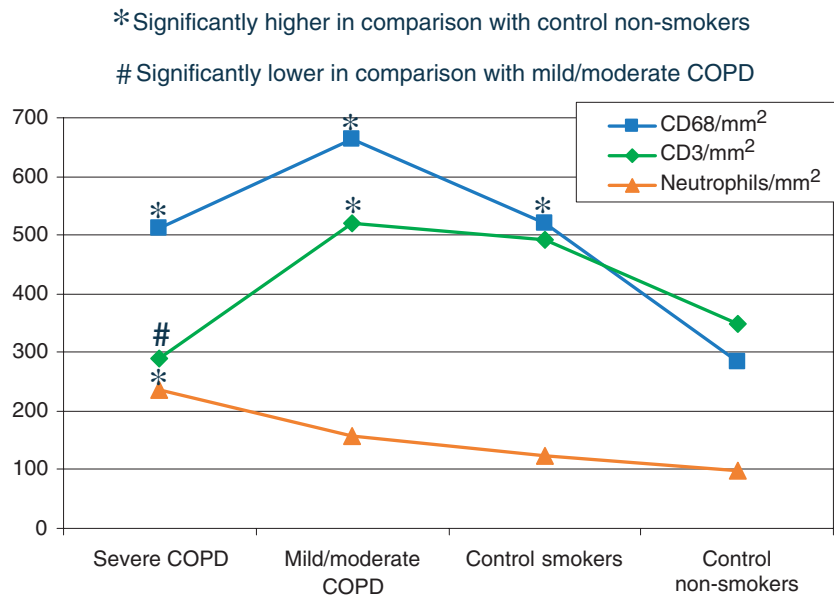


Fig. 2. Schematic representation of the cellular variations of CD68⁺, CD3⁺ and neutrophils (cells/mm²) in the bronchial biopsy submucosa of control non-smokers, control smokers, mild/moderate and severe chronic obstructive pulmonary disease (COPD) patients. Macrophages (CD68⁺ cells) are significantly increased in smokers and in both subgroups of patients with COPD (mild/moderate and severe) in comparison with control non-smokers. T lymphocytes are increased in the mild/moderate forms of the disease in comparison with control non-smokers, but their number decreases significantly with worsening of the disease. Neutrophils increase progressively in number from control non-smokers to severe COPD patients, where they reach the highest values in the bronchial biopsies. Graph based on references [10, 11, 21].

Table 4. Cascade of events in the bronchial mucosa from control non-smokers to severe COPD

Control non-smokers

In the normal subjects bronchial epithelium shows a relatively low expression of ICAM-1, NF- κ B, STAT-4 and IL-8*

In the submucosa the number of T lymphocytes and macrophages is relatively low and occasionally can be observed in the presence of neutrophils and eosinophils. Little expression of endothelial ELAM-1 is observed. The presence of activated T cells expressing NF- κ B, T cells and macrophages expressing STAT-4 and IFN- γ as well as activated neutrophils expressing MPO is relatively low.

Control smokers

In 'healthy' smokers bronchial epithelium shows similar features of that reported in normal subjects

In the submucosa the number of CD3⁺ and CD8⁺ cells is increased even though their activation state is slightly or not changed. Numbers of NF- κ B⁺ cells (including CD4⁺ and CD8⁺ cells) is increased in concomitance with increased T cell infiltration. Endothelial activation (ELAM-1 overexpression) as well as the presence of activated neutrophils (MPO⁺ cells) and eosinophils (EG-2⁺ cells) is in the range of normal subjects

Mild/moderate COPD

In mild/moderate disease bronchial epithelium shows increased expression of ICAM-1, NF- κ B, STAT-4 and IL-8*

In the submucosa the number of CD3⁺, CD8⁺ and CD68⁺ cells is increased as well as numbers of activated T cells (both CD4 and CD8) and macrophages expressing NF- κ B, STAT-4 and IFN- γ proteins. Endothelial ELAM-1 is increased in association with increased BAL and sputum neutrophilia. A rapid migration of neutrophils through the bronchial mucosa has been postulated. The number of total and activated neutrophils and eosinophils in the bronchial tissue is slightly or not changed

Severe COPD

In severe disease bronchial epithelium shows increased expression of MIP-1 α and MPO. We can argue that overexpression of ICAM-1, IL-8* and of transcription factors is maintained even though specific data are lacking

In the submucosa the number of CD3⁺, CD8⁺ and activated CD3⁺ coexpressing CCR5 receptor is decreased. The number of CD68⁺ cells remains elevated. The number of total and activated (MPO⁺ cells) neutrophils increases in concomitance with increased nitrotyrosine immunoexpression. ELAM-1 remains elevated in concomitance with the increased tissual neutrophilia. Epithelial and endothelial activation may account for increased neutrophilic and macrophagic migration in the bronchial tissue. In turn, peroxynitrite formation via MPO reacting with nitrites (NO₂⁻) may cause tissual damage followed by small pro-inflammatory peptides release and increased adhesion of neutrophils and macrophages. Since IL-8 induces release of MPO from neutrophils, IL-8 secreted by bronchial epithelium, neutrophils themselves and macrophages may further substatin this inflammatory process

ICAM-1, intercellular adhesion molecule-1; NF- κ B, nuclear factor-kappa B; STAT-4, signal transducer and activators of transcription; ELAM-1, endothelial adhesion molecule-1; MPO, myeloperoxidase; COPD, chronic obstructive pulmonary disease.

*Reported from primary cultured bronchial epithelial cells [31].

recruitment and increased survival (i.e. IL-5 and ICAM-1 increase) were not changed in mild/moderate COPD during exacerbations [14]. We suggest that the further increase of the 'stable' component of inflammatory cells in the bronchial

mucosa of exacerbated COPD, constituted by T lymphocytes and neutrophils and activation of macrophages and TNF- α overproduction [13], may play a prominent role in the exacerbation process and related functional changes.

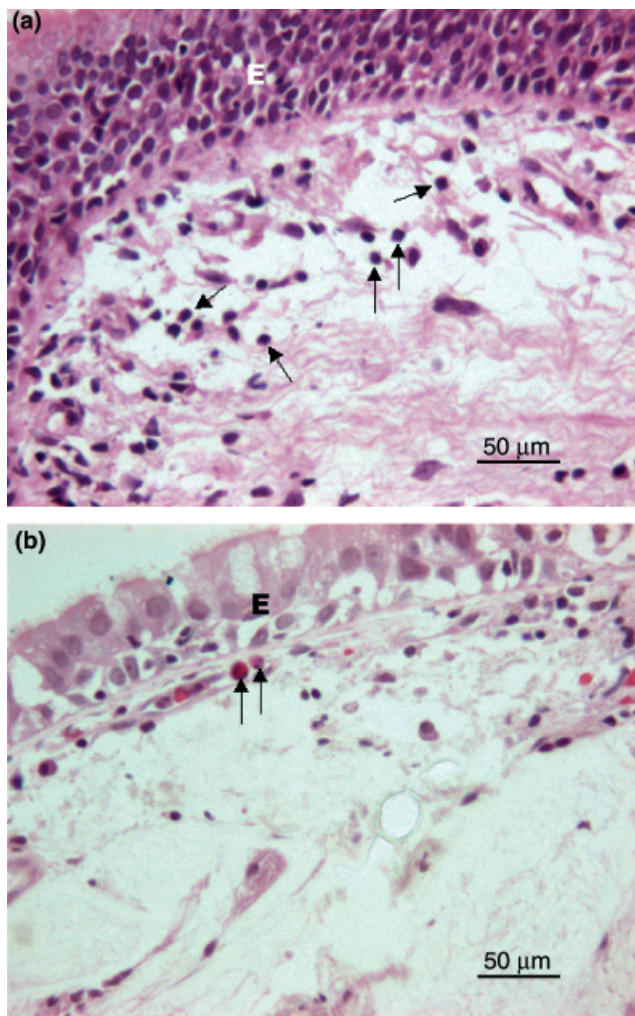


Fig. 3. Photomicrographs showing the bronchial biopsies of patients with (a) stable mild/moderate chronic obstructive pulmonary disease (COPD) and (b) exacerbated mild/moderate COPD stained with haematoxylin & eosin for identification of the total inflammatory cell infiltration and eosinophils in the bronchial mucosa. In stable mild/moderate COPD (a) the mononuclear cell infiltration predominates. The thickness of the reticular basement membrane is generally normal. In exacerbated mild/moderate COPD (b) the appearance of eosinophils is a frequent observation. Eosinophils in exacerbated COPD are frequently confined to the capillary vessels of the bronchial mucosa and, at variance with asthma, their presence is transitory and they are poorly or non-degranulated. Original magnification: $\times 40$; arrows indicate mononuclear cells (a) and non-degranulated eosinophils (b). E, epithelium.

Lung parenchyma

Lung surgical specimens in stable chronic obstructive pulmonary disease

Central, peripheral airways and alveolar septa While bronchial biopsies are generally obtained from volunteers or from patients with a diagnostic indication for bronchoscopy coming from the general population of COPD patients or from control groups, lung surgical specimens are most frequently obtained from subjects undergoing lung resection for peripheral carcinomas or from subjects with emphysema undergoing LVRS. In both cases, selection criteria of the resected lung tissue may create important biases in the analysis of inflammation of the airways and parenchyma. Inflamma-

tion in lungs of patients with lung carcinoma, as determined by BAL analysis, is either increased [51] or unchanged [52] compared with lungs without lung carcinoma. A decreased [53] or increased [54] activity of natural killer (NK) cells has been reported in lungs of patients with lung carcinoma. These data suggest that a variable immunological response develops, which in turn may influence the results when these tissues are used for analysis of inflammatory and immunological responses in different lung diseases such as COPD. Furthermore, subjects selected for LVRS represent a limited subgroup of COPD patients suffering prevalently from emphysema. Notwithstanding these limitations, however, important results on the inflammatory process have been obtained in the central, peripheral airways and lung parenchyma of patients with COPD and control subjects.

Central airways Total inflammatory cell infiltration is significantly increased in the central airways of patients with chronic bronchitis with normal lung function compared with control smokers [26, 55] (Table 2). In the central airways and submucosal glands of chronic bronchitics, IL-4 but not IL-5 was also reported to be increased in association with the increased mucus production [26]. These data support the hypothesis that inflammation plays an important role in the induction of hypersecretion of mucus glands.

In the central airways of mild/moderate COPD patients, total inflammation was reported to be decreased compared with chronic bronchitics with normal lung function [26], or not changed compared with control smokers [26, 56] (Table 2). Neutrophils, macrophages and T lymphocytes were not changed in comparison with control smokers [26, 56] (Table 2).

Peripheral airways Total inflammatory cell infiltration has been reported as significantly increased in peripheral airways (<3 mm diameter) of patients with chronic bronchitis with normal lung function compared with control smokers [57] (Table 2). Since the glandular tissue is mainly confined to the larger airways (>3 mm diameter), the increased total inflammation observed in peripheral airways of chronic bronchitics may have a different action from that reported in the central airways (increased mucous hypersecretion) of these patients [58]. This may favour the increased airway wall thickening, collagen deposition and smooth muscle hypertrophy, when bronchial obstruction develops in the peripheral airways of these patients [59].

Conflicting results have been reported in the peripheral airways of mild/moderate COPD patients. Saetta et al. [60] showed increased total inflammation and CD8⁺ cells in comparison with control smokers, but no significant changes were reported by others [61–63]. Similar contrasting results have been reported for macrophages [60–63]. Neutrophils and CD4⁺ cells were reported not changed in mild/moderate COPD patients compared with control smokers [60–63].

These data show that an increased inflammatory cell infiltration is evident in central and peripheral airways of patients with chronic bronchitis and near-normal lung function. In contrast, the presence of airflow limitation at a mild/moderate level is associated with a relatively lower number of mononuclear cells infiltrating both central and peripheral airways. Parallel to this reduction, indices of fibrosis and of smooth muscle thickening increase, as also

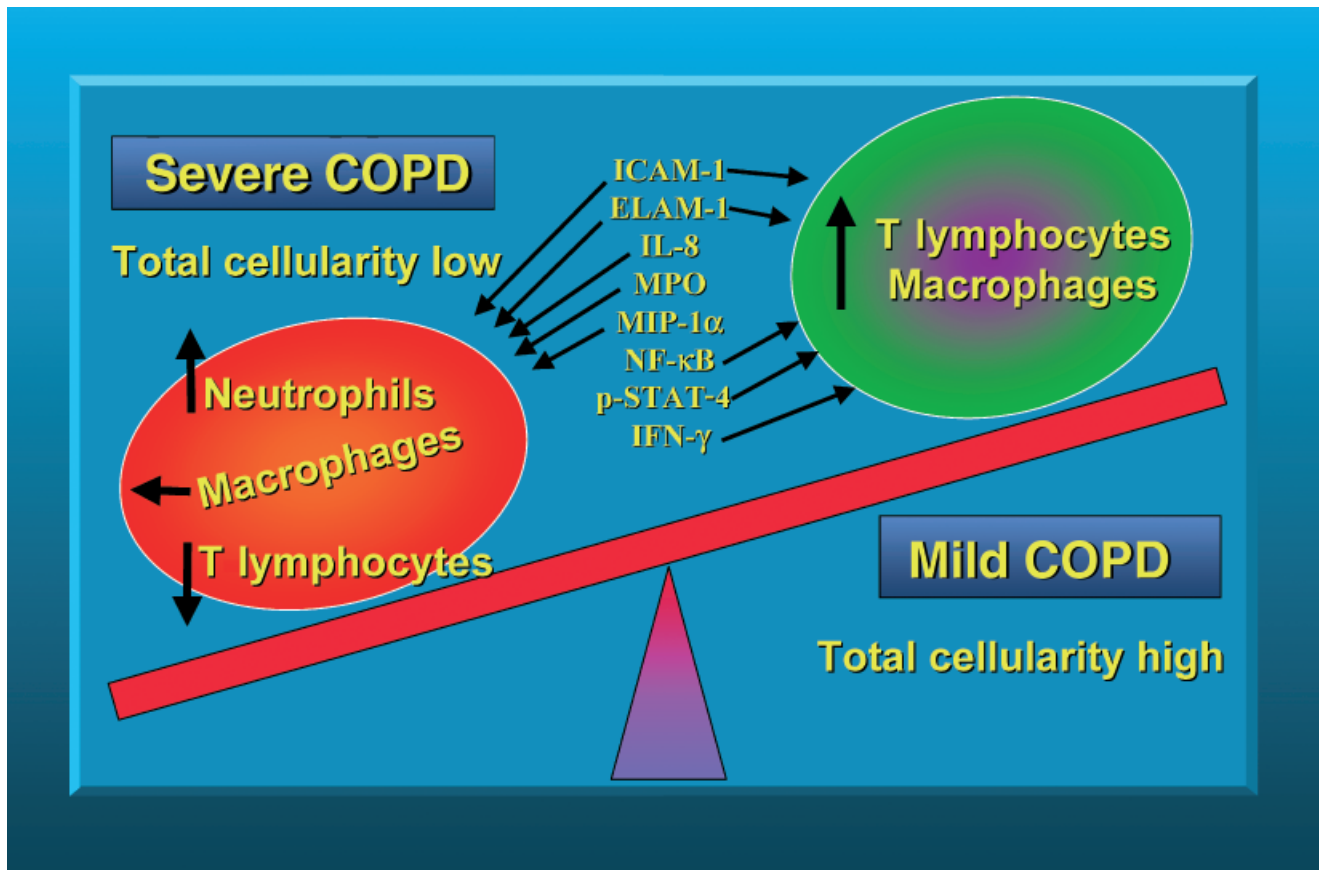


Fig. 4. General scheme showing the cellular prevalence and the pro-inflammatory molecules involved in this cellular migration and activation in mild and severe chronic obstructive pulmonary disease. ICAM-1, intercellular adhesion molecule-1; ELAM-1, endothelial adhesion molecule-1; MPO, myeloperoxidase; MIP-1 α , macrophage inflammatory protein-1 α ; NF- κ B, nuclear factor- κ B; p-STAT-4, phospho-signal transducer and activator of transcription-4.

documented with a semi-quantitative approach by Bosken et al. [59]. The molecular mechanisms inducing airway fibrosis and smooth muscle thickening need to be better evaluated.

In mild/moderate COPD, conflicting results have been reported for TGF- β ₁, a pro-fibrotic protein mainly secreted by epithelial cells, in comparison with control smokers [63, 64] (Table 2). Furthermore, the CXCR-3 chemokine receptor expression, indicative of a Th1/Tc1 immunological response, has been reported to be increased in comparison with control non-smokers [65]. In patients with a similar degree of airway obstruction, increased MCP-1, IL-8 and macrophage infiltration has also been reported in the bronchiolar epithelium in comparison with control smokers [42].

These data, taken together, may suggest a differential inflammatory cell response and remodelling of the peripheral and central airways in COPD, with decreased mononuclear cell infiltration and increased structural changes (i.e. fibrosis) developing early in the peripheral airways of mild/moderate diseased patients followed by similar modifications in the central airways in the worsened disease, as testified by our data based on bronchial biopsies from patients with severe COPD [10, 11]. Anatomical differences between peripheral and central airways, showing different muscular and micro-vascular components, may contribute, however, to these differences in inflammatory cell prevalence.

One study, performed in the peripheral airways of patients with severe COPD after LVRS showed a further increase of

CD4⁺, CD8⁺ cells and of total leucocytes (CD45⁺ cells) in comparison with mild/moderate disease, without changes in the numbers of neutrophils, mast cells or eosinophils [66]. Similar results were seen in the lung parenchyma of severely diseased patients [67]. Lung specimens obtained at LVRS for emphysema, however, are generally more inflamed than those obtained from lungs with localized tumours [61–63, 67] or from bronchial biopsies [10, 11]. Comparative studies, specifically designed to evaluate inflammatory changes in specimens from LVRS and lung tumours, involving patients matched for age, degree of airway obstruction and smoking habit, need to be carried out to clarify this issue. Further studies are also needed to define the inflammatory picture that predominates in the bronchial tree (central and peripheral airways) of severely diseased COPD patients.

Lung parenchyma and alveoli Lung parenchyma analysis tends to be carried out either on specimens obtained from lungs with localized tumours, in the case of mild/moderate COPD patients or control groups, or on specimens obtained at LVRS for emphysema, in the case of severe COPD. Nevertheless, interesting results, which merit review, have been obtained.

Smokers with normal lung function showed increased numbers of macrophages and T lymphocytes in the lung parenchyma compared with control non-smokers [68], without changes in CD4⁺, CD8⁺, B cells and eosinophils [69, 70]

(Table 3). In mild/moderate COPD patients, increased CD8⁺ cells were reported in the alveolar septa compared with control non-smokers [70, 71], with no changes in the numbers of neutrophils, macrophages, CD4⁺ cells or eosinophils [70, 71] (Table 3). In patients with similar disease severity, TGF- β 1 was increased in bronchiolar and alveolar epithelium compared with control smokers [72] (Table 3). Conflicting results have been reported for γ -glutamyl cysteine synthetase (γ -GCS), a protective enzyme of alveolar epithelium against oxidants: γ -GCS in the alveolar epithelial cells of mild/moderate COPD patients was increased compared with control smokers in one study [72] but not in another [73].

Few data are available on severe COPD patients. Retamales et al. [69] showed greater levels of total lung parenchymal inflammation in severe disease constituted by a greater presence of neutrophils, macrophages (CD68⁺), CD4⁺, CD8⁺ cells and eosinophils compared with mild/moderate COPD and control smokers. In specimens obtained in a way similar to that reported by Retamales et al. deoxyribonucleic acid (DNA) fragmentation and TUNEL positivity were reported to be increased in alveolar septa of severe emphysematous lungs as compared with control smokers and non-smokers [74]. A parallel decrease of vascular endothelium growth factor (VEGF)-A and VEGF receptor (VEGF-R)-2 was also reported in this study. The authors propose that an increased apoptosis of the endothelial cells in the alveolar septa triggered by a lack of growth factors (VEGF) and its main receptor may play a role in the development of pulmonary emphysema [74]. Imai et al. [75] showed that matrix metalloproteinase (MMP)-1 but not MMP-9 or MMP-12 was significantly increased in the alveolar septa of patients with severe emphysema compared with control non-smokers. MMP-1 was mainly localized in the type II pneumocytes of emphysematous lungs. The authors speculate that different enzymes such as elastase and MMP-12 may play a role in the early stages of the disease, while collagen destruction by collagenase-1 (MMP-1) seems to be the prevalent feature in the more severe forms of the disease [75]. This speculation is supported by a recent study showing that MMP-12 and neutrophils are required for acute cigarette smoke-induced connective tissue breakdown, and that this result is mediated by increased TNF- α release from alveolar macrophages, up-regulation of NF- κ B, endothelial activation and increased neutrophil influx in the lungs of mice [76].

Few studies have directly compared mild and severe emphysema [77, 78]. Decreased peribronchiolar decorin, biglycan and alveolar heparan sulphate proteoglycan (HSPG) have been found in severe compared with mild emphysema [77] without changes of collagen I, III, IV, laminin and fibronectin immunoreactivity between the two groups [77]. The same group of authors showed decreased percentages of nitric oxide synthase (NOS)2 or NOS3⁺ alveolar macrophages in severe compared with mild emphysema [78]. The authors suggested that alterations of proteoglycans, subsequent to fibrillar collagen stabilization, may play a role in the development of severe emphysema [77]. Increased NO production, related to the increased percentage of NOS2⁺ and NOS3⁺ macrophages, seen in mild compared with severe emphysema, may have a protective function in mild disease in response to increased inflammation due to cigarette smoke exposure [78].

Conflicting results have been reported for collagen synthesis in emphysematous lungs [79, 80]. Elastin expression was decreased in panacinar and severe centriacinar emphysema [80]. A more recent report, performed in mild/moderate emphysema, showed that alveolar septa close to the emphysematous areas are thickened and produce more elastin and collagen [81], though it is not known whether these newly synthesized molecules maintain their original functional status [81].

These data, taken together, suggest that T lymphocytes may play a role in the alveolar septa disruption in mild emphysema and in the mild/moderate airflow obstruction related to emphysema. Severe emphysema seems to be accompanied by a general increase of alveolar inflammatory cells including neutrophils, macrophages and T lymphocytes, increased indices of alveolar apoptosis and collagenase-1, and decreased alveolar expression of VEGF-A, VEGFR-2 and proteoglycans. More studies comparing severe vs. mild forms of emphysema are needed to clarify the prevalent molecular mechanisms involved in the progression of the disease.

The data summarized in Tables 2 and 3 on central/peripheral airways and lung parenchyma were obtained from lung tissue resected for localized lung tumours and were focussed on mild/moderate COPD and control groups.

Concluding comments

In the last decade, analysis of bronchial biopsies of COPD patients and control subjects has yielded specific information on the role of inflammation in the pathogenesis of this disease. T lymphocytes, in particular CD8⁺ cells and macrophages, are the prevalent inflammatory cells in the bronchial mucosa of all smokers with and without mild airflow limitation, while neutrophils and macrophages predominate in the most severe forms of COPD. The molecular mechanisms causing the prevalence of T lymphocytes and neutrophils, respectively, in mild and severe disease need to be extensively 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 in number and functions need to be studied in more detail. Through analysis of bronchial biopsies, it is possible to evaluate inflammation in different clinical conditions, such as during an exacerbation period of the disease or after pharmacological treatment. Even though relatively few studies are published on these two latter conditions, a preliminary picture is today available. A general increase of inflammatory cells and the transient appearance of eosinophils was reported in exacerbations of mild/moderate COPD, while a 3-month glucocorticoid treatment of stable moderate COPD patients had little effect on the presence of inflammatory cells in bronchial biopsies. Follow-up studies involving the same group of patients before and after treatment are needed to better evaluate the cellular changes after treatment. Moreover, the effect of an anti-inflammatory treatment in the bronchial biopsies from mild stable COPD patients needs to be investigated. Data summarized in this review regarding peripheral airways and parenchyma of mild/moderate COPD and controls were obtained from resected

lungs for localized tumours. Alterations in severe emphysema, here reported, were studied in specimens obtained at LVRS from patients with emphysema. Whether these latter patients are really representative of the entire population of severely diseased COPD patients needs to be confirmed.

Conflicting results have been reported in the peripheral airways of mild/moderate COPD patients, although in the mildest forms of the disease an increased mononuclear cell infiltration is documented, in agreement with observations on bronchial biopsies. We believe that the greater variability of the results obtained in peripheral airways of COPD patients may be the consequence of variable inflammatory states described in the lungs of patients suffering from lung cancer, or, alternatively, due to the development of structural modifications such as bronchial fibrosis and smooth muscle hypertrophy, with a concomitant reduction of the total inflammatory cell infiltration, which occurs early in the peripheral airways of patients with mild/moderate COPD and extends later to the central airways.

The analysis of inflammation in the lung parenchyma of mild/moderate COPD yields similar results and suffers from the same constraints reported for peripheral airways. Guidelines for recruitment of patients with COPD undergoing bronchoscopy and bronchial biopsy collection, as well as lung resection for tumours or LVRS for emphysema, would be useful to obtain a more widespread application of standardized selection criteria.

While the inflammatory cell picture of the central/peripheral airways and lung parenchyma in mild/moderate COPD is becoming clearer, the analysis of the molecular mechanisms underlying COPD development has only just begun. Current data regarding the molecular alterations in bronchial biopsies, peripheral airways and alveoli are fragmentary and should be considered as preliminary observations warranting further confirmation. A clear, organic picture in the lung pathology concerning the type of cytokine prevalence, oxidant/anti-oxidant balance, proteinase-anti-proteinase imbalance and the molecules involved in inducing the structural alterations in the lung of COPD patients in different clinical conditions (stable, exacerbated, glucocorticoid-treated disease) and with different degrees of severity (from mild-to-severe forms) needs to be adequately defined through a quantitative approach.

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