Cellular and Structural Bases of Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is defined as a disease state characterized by poorly reversible airflow limitation that is usually both progressive and associated with an abnormal inflammatory response of the lung (1). Cigarette smoking is the most important risk factor for the development of COPD. However, only a minority of smokers develop COPD and the reason is still unknown.

The pathological hallmarks of COPD are inflammation of the peripheral airways and destruction of lung parenchyma or emphysema. The functional consequence of these abnormalities is expiratory airflow limitation. Since the major determinants of expiratory flow are a driving pressure that promotes flow (elastic recoil of the lung) and an opposing resistance that inhibits flow (airway obstruction), the reduction in flow occurring in COPD is more correctly defined as airflow limitation rather than airflow obstruction, since both loss of elastic recoil and increase in airway resistance play an important role in the observed decrease in flow. Emphysema will contribute to the airflow limitation by reducing the elastic recoil of the lung through parenchymal destruction, as well as by reducing the elastic load applied to the airways through destruction of alveolar attachments. On the other hand, inflammation of the peripheral airways will contribute to the airflow limitation by increasing the thickness of the airway wall which, together with fibrosis and smooth muscle hypertrophy, may cause airway narrowing. The role of mucus hypersecretion in the development of chronic airflow limitation is still controversial (2, 3). The main site of mucus hypersecretion, expressed clinically as chronic bronchitis, is the central airways, and there is increasing evidence that the central airways are inflamed in patients with COPD.

Pulmonary hypertension is a common feature in patients with advanced COPD, but the precise mechanisms of increased vascular resistance are unclear. For many years, it has been regarded as a consequence of the hypoxic vasoconstriction that may occur in advanced stages of the disease. However, the lack of reversibility of pulmonary hypertension after hypoxemia correction suggests that it might be due at least in part to the development of pulmonary vascular inflammation and remodeling (4–6).

In summary, in subjects with COPD, pathological changes can be found in the central airways, the peripheral airways, the lung parenchyma, and pulmonary arteries. Interestingly, some of these changes can already be present in the lungs of "normal" smokers, i.e. smokers with normal lung function, indicating that smoking itself is able to damage the lung even before airflow limitation occurs. In the present article we will focus on the cellular and structural changes present in the lungs of "normal" smokers and on those present in the lungs of smokers with COPD, in an attempt to underline the possible mechanisms contributing to airflow limitation in these patients. We will then review the few studies that described the cellular and structural changes that occur in severe COPD and those that occur during an exacerbation of the disease. Finally, we will address the effect of smoking cessation or antiiflammatory treatment in an attempt to investigate the potential reversibility of the pathologic lesions characteristic of COPD.

In advanced COPD, changes in the right heart, the respiratory muscles, and the skeletal non-respiratory muscles as well as cachexia may also occur, but these systemic changes will not be discussed in this article.

"NORMAL" SMOKERS

It is now well accepted that cigarette smoking can elicit an inflammatory reaction involving the entire tracheobronchial tree even in the absence of an established airflow limitation (7, 8).

Studies examining central airways in smokers have shown that T-lymphocytes and macrophages are the predominant cells infiltrating the airway wall, whereas neutrophils, that are scanty in the airway wall, are increased in the airway lumen (7, 9) (Table 1). This discrepancy has led to the hypothesis that the inflammation in the lumen may be different from that in the bronchial wall of smokers. However, a possible explanation for this discrepancy could be the rapid migration of neutrophils across the tissue and their accumulation into the lumen so that, at any time point, these cells can be detected more easily in the bronchial lumen than in the bronchial wall.

Studies examining peripheral airways in smokers are particularly relevant because, as elegantly demonstrated by the pioneering work of Hogg and coworkers, peripheral airways are the major site of increased resistance in smokers (10), and therefore early lesions in this zone of the lung may have important functional consequences. Niewohener and coworkers (8) were the first to demonstrate that an inflammatory reaction is already present in the peripheral airways of young smokers who experienced sudden death outside the hospital, supporting the idea that early structural changes may occur in peripheral airways of smokers before COPD is established. These early lesions included an inflammatory cell infiltrate in the airway wall consisting predominantly of mononuclear cells and clusters of macrophages in the respiratory bronchioles. Interestingly, the authors reported that these lesions were

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present in the absence of noteworthy tissue destruction and fibrosis, and suggested that this stage of the disease could still be largely reversible (Table 1).

SMOKERS WITH ESTABLISHED COPD

In smokers, the development of airflow limitation is associated with cellular and structural changes in both peripheral and central airways. In peripheral airways these changes include airway wall inflammation, fibrosis, smooth muscle hypertrophy, goblet cell metaplasia and lumen occlusion by mucous plugging (11–13) (Table 1). Inflammation, fibrosis and smooth muscle hypertrophy, by increasing the thickness of the airway wall, may facilitate uncoupling between airways and parenchyma and promote airway narrowing. In addition, airway wall inflammation could contribute to the destruction of alveolar walls which attach to the airways, allowing the airway wall to deform and narrowing the airway lumen (14). Goblet cell metaplasia may produce an excess of mucus which could obstruct the lumen and alter the surface tension of the airway lining fluid, rendering the peripheral airways unstable and facilitating their closure (13). Despite the fact that airway wall fibrosis could be a major contributor to the irreversible component of airflow limitation in smokers with COPD, a precise characterization of the fibrotic tissue present in peripheral airways of these subjects has never been reported.

In central airways, the development of airflow limitation is associated with a further increase of macrophages and T lymphocytes in the airway wall and of neutrophils in the airway lumen (9, 15, 16) (Table 1), suggesting a selective passage of neutrophils across the epithelium into the airway lumen.

Although the mechanism of neutrophil accumulation into the airway lumen in smokers with COPD is not entirely clear, it is possible that an imbalance between pro- and anti-inflammatory cytokines may play a role. Interleukin-10 (IL-10), a cytokine that reduces inflammatory responses, is decreased in the airway lumen of smokers with COPD (17), whereas IL-8, a cytokine that promotes neutrophil chemotaxis, and Tumor Necrosis Factor (TNF)-α, a cytokine that activates adhesion molecules, are increased (9). The observation of an upregulation of the adhesion molecules E-selectin and ICAM-1 on submucosal vessels and on bronchial epithelium of smokers with COPD (18) suggests a mechanism for recruitment of neutrophils from the circulation and for their migration into the airway lumen through the epithelium. The finding of an increased number of neutrophils in the bronchial epithelium of smokers with COPD supports this hypothesis (19). Neutrophils are also increased in the bronchial glands of these subjects (19), and this location may be crucial for the development of mucus hypersecretion in COPD, since neutrophil elastase is a remarkable potent secretagogue. Although for many years mucus hypersecretion has been considered to be irrelevant to the development of chronic airflow limitation in smokers (2), a recent study has shown that chronic sputum production was significantly associated with both an excess of FEV_1 decline and an increased risk of subsequent hospitalization because of COPD, supporting a role for mucus hypersecretion in the development of chronic airflow limitation (3).

One of the major characteristics of airflow limitation in COPD is that it is progressive, but longitudinal studies assessing the lung pathology in subjects with an accelerated decline in lung function are lacking. In a 15-year follow-up study Stanescu and colleagues found that, in smokers, the accelerated decline in lung function was associated with an increased number of neutrophils in the airway lumen (20). In addition, in subjects with a more rapid decline in FEV_1, neutrophils exhibited an increased expression of the adhesion molecule CD11b/CD18, the ligand for ICAM-1. The correlation observed between increased expression of CD11b/CD18 and reduced expiratory flow in these subjects (21) provides further evidence for the role of adhesion molecules in COPD.

Although the airway lumen in smokers with COPD displays a neutrophilic inflammation, the airway wall in these subjects shows an increase in macrophages and T-lymphocytes (15). In addition, there is a shift in the balance of the CD4/CD8+ve T-lymphocyte ratio in favor of the CD8 (12, 16) (Table 1). Indeed, CD8+ve cytotoxic T lymphocytes infiltrate the central airways (16), the peripheral airways (12) and the lung parenchyma (4), suggesting a consistent inflammatory process along the entire tracheobronchial tree in smokers with COPD

TABLE 1. CELLULAR AND STUCTURAL CHANGES PRESENT IN THE LUNGS OF "NORMAL" SMOKERS AND OF SMOKERS WITH ESTABLISHED COPD

	"Normal" smokers	Smokers with established COPD
Central airways		
Wall	• T-lymphocytes • Macrophages	• Further incease in macrophages and T-lymphocytes (particularly $CD8^{+ve}$ T-lymphocytes) • Neutrophils in severe disease
Lumen	• Neutrophils	• Neutrophils
Peripheral airways	• Mononuclear cells • Clusters of macrophages in the respiratory bronchioles	• Goblet cell metaplasia and mucous plugging • Smooth muscle hypertrophy • Fibrosis • Inflammation (particularly $CD8^{+ve}$ T-lymphocytes) • All inflammatory cells including neutrophils in severe disease
Parenchyma	• No destruction • No fibrosis	• Inflammation (particularly $CD8^{+ve}$ T-lymphocytes) • Destruction (centriacinar and panacinar emphysema) • Fibrosis
Pulmonary arteries	• Intimal thickening	• Endothelial dysfunction • Intimal thickening • Medial thickening (less frequently) • Adventitial inflammation (particularly $CD8^{+ve}$ T-lymphocytes)

(Table 1). Interestingly, CD8+ve cytotoxic T lymphocytes not only are increased in number in all these lung compartments, but also showed a significant correlation with the degree of airflow limitation (4, 12, 16), suggesting a role for these cells in the progression of the disease (Figure 1).

Traditionally, the major activity of CD8+ve cytotoxic T-lymphocytes has been considered the rapid resolution of acute viral infections, and viral infections are a frequent occurrence in patients with COPD. The observation that people with frequent respiratory infections in childhood are more prone to develop COPD supports the role of viral infections in this disease (22). It is conceivable that, in response to repeated viral infections, an excessive recruitment of CD8+ve cytotoxic T-lymphocytes may occur and damage the lung in susceptible smokers, possibly through the release of TNFα (23). On the other hand, it is also possible that CD8+ve cytotoxic T-lymphocytes are able to damage the lung even in the absence of a stimulus such as viral infection, as shown by Enelow and coworkers (24), who clearly demonstrated that recognition of a lung "autoantigen" by T cytotoxic cell may directly produce a marked lung injury. Taking into account these findings, it can be hypothesised that the cytotoxic T cell accumulation observed in COPD could be a response to an "autoantigenic" stimulus originating in the lung and induced by cigarette smoking. The observation that CD8+ve T lymphocytes are increased not only in the airways, but also in the lung parenchyma, of smokers with COPD (4) invites speculation that these cells, because of their location within the alveolar walls, may contribute to the development of parenchymal destruction that characterizes emphysema.

The pathogenesis of parenchymal destruction in emphysema remains enigmatic, although the proteases-antiproteases imbalance hypothesis is widely supported. Briefly, the concept is that activated inflammatory cells release elastases which destroy the lung tissue, overwhelming local antiprotease activities. The major sources of elastases in the lung are polymorphonuclear cells and macrophages, and their products include leukocyte elastase, proteinase 3, matrix metalloproteinases, cystein proteinases and plasminogen activators, all substances potentially capable of destroying the lung parenchyma. However, since many cigarette smokers and patients with severe inflammatory lung parenchymal diseases (like pneumonia and adult respiratory distress syndrome) do not develop significant emphysema, this hypothesis may not fully explain the loss of lung tissue in cigarette smoking-induced emphysema (25).

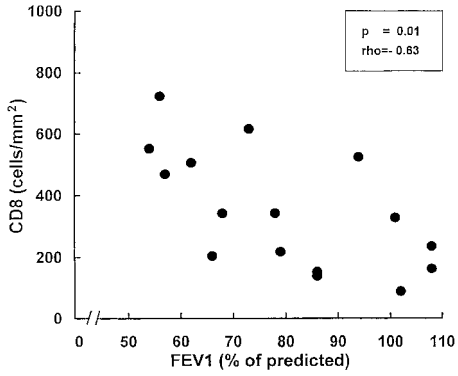

Figure 1. Relationship between the number of CD8+ve T-lymphocytes infiltrating the peripheral airways and values of FEV_1 in smokers. From Reference 12.

An interesting alternative mechanism has been recently proposed by Kasahara and coworkers (25). They hypothesized that the disappearance of lung tissue in emphysema may involve accelerated apoptosis of endothelial and epithelial cells. The authors did indeed experimentally demonstrate that chronic blockade of vascular endothelial growth factor receptors is able to induce alveolar septal cell apoptosis and emphysema, supporting the role of apoptosis in the pathogenesis of the disease.

Emphysema is defined anatomically as a permanent destructive enlargement of air spaces distal to the terminal bronchioles, without obvious fibrosis. The destructive process can be detected microscopically in the alveolar walls of smokers even when there is no evidence of airspace enlargement (26). The microscopic measurement of this parenchymal destruction can, therefore, allow an early identification of the disease, at a time when emphysema is not detectable macroscopically. The functional significance of such early destruction is demonstrated by its correlation with indices of airflow limitation and loss of elastic recoil of the lung. In contrast with the above definition, recent data have shown that the destructive process is accompanied by a net increase in the mass of collagen, suggesting that there is active alveolar wall fibrosis in emphysematous lungs (27) (Table 1).

Smokers can develop two main morphological forms of emphysema that can be distinguished according to the region of the acinus which is destroyed (28) (Table 1). Centriacinar (or centrilobular) emphysema is characterized by focal destruction restricted to respiratory bronchioli and the central portions of the acinus, surrounded by areas of grossly normal lung parenchyma. This form of emphysema is usually most severe in the upper lobes of the lung. Panacinar (or panlobular) emphysema is characterized by destruction of the alveolar walls in a fairly uniform manner, i.e., all the air spaces beyond the terminal bronchiole are involved. The panacinar form is characteristic of patients who develop emphysema relatively early in life, and, in contrast to the centriacinar form, has a tendency to involve the lower lobes more than the upper. The familial form of panacinar emphysema is usually associated with deficiency of α_1-antitrypsin, which normally protects the respiratory region by forming a highly effective anti-elastase screen.

The two forms of emphysema have distinct mechanical properties and distinct peripheral airway involvement (28). In particular, the lung compliance is greater in panlobular than in centrilobular emphysema, whereas the extent of peripheral airway inflammation is greater in the centrilobular than in the panlobular form. It is possible that, in centrilobular emphysema, airflow limitation is primarily a function of peripheral airway inflammation, as supported by the correlation between reduced expiratory flow and increased airway inflammation observed in this form of emphysema. By contrast, in panlobular emphysema, airflow limitation seems to primarily be a function of loss of elastic recoil, as supported by the correlation between reduced expiratory flow and increased compliance observed in this form of emphysema (28).

SMOKERS WITH SEVERE COPD

There are very few studies that investigated the lung pathology in severe COPD, and these studies demonstrated an increase of all inflammatory cells, including neutrophils (29, 30). As airflow limitation progressively worsens, neutrophils in the bronchial wall increase, and their increase is correlated with the degree of airflow limitation (30). Interestingly, an association between neutrophilia and severity of disease has recently

been reported in asthma as well (31), suggesting a role for these cells in the progression of both asthma and COPD.

Surprisingly, autopsy studies on subjects with severe COPD are very few. The largest study, performed by Nagai and colleagues (32), showed that these subjects had both emphysema and peripheral airway abnormalities. Although the relative role of each of these pathologic lesions in the development of airflow limitation was difficult to establish, the authors concluded that emphysema had the most important role. However, as suggested by Snider (33), the findings of Nagai and colleagues must be cautiously interpreted. Their data indicate that, when emphysema is severe, loss of elastic recoil assumes overwhelming importance as a mechanism of airflow limitation, thus masking the effects of peripheral airway abnormalities. By contrast, when emphysema is mild, peripheral airway abnormalities do appear to play a role in causing airflow limitation.

A common feature in patients with severe COPD is pulmonary hypertension, which represents a major predictive factor of hospitalization for acute exacerbation of the disease (34) and is associated with a shorter life expectancy (35). Potential causes of pulmonary hypertension in COPD include hypoxic pulmonary vasoconstriction, emphysematous destruction of the capillary bed, and remodelling of pulmonary arteries. In pulmonary arteries of subjects with COPD the most consistent morphological change is the thickening of the intimal layer produced by the proliferation of smooth muscle cells and by the deposition of both elastic and collagen fibers (5, 6). Less frequently, some authors reported a moderate degree of muscular hypertrophy in the medial layer (6). Recently, an infiltration of CD8+ve cytotoxic T-lymphocytes has been demonstrated in the adventitial layer (4, 5) (Table 1). These structural changes are often associated with functional abnormalities of the endothelium, and in particular with an impaired release of endothelium-derived relaxing factors (36). The recent observation that intimal thickening may also be present in smokers with mild COPD as well as in smokers with normal lung function, who are not hypoxemic (37), suggests that factors other than hypoxemia may play a role in the development of structural changes in pulmonary arteries of smokers with COPD (Table 1).

EXACERBATIONS

Smokers with COPD are prone to acute exacerbations, defined on clinical grounds as increased dyspnea, cough, and sputum production that cause the subject to seek medical attention. Despite the fact that exacerbations represent an important feature of the clinical manifestation and natural history of COPD, they are not included in the definition of the disease. Moreover, although exacerbations are a common cause of visits to general practitioners, accesses to emergency departments, and hospital admissions, their mechanism is still unknown. The role of bacterial infections, once believed to be the main cause of COPD exacerbations, is now debated since it is evident that many exacerbations in COPD are due to other causes such as viral infections and environmental factors (38).

Patients with COPD examined during a mild exacerbation of the disease showed a prominent eosinophilia both in the airway wall and in the airway lumen (39) (Figure 2). Although the cause of this eosinophilia is unknown, viral infections may have a role since respiratory viruses are able to stimulate the production of eotaxin, a potent eosinophil chemoattractant (40). In more severe patients, who had an exacerbation of bacterial origin, myeloperoxydase, a marker of neutrophil activation, and IL-8, a potent neutrophil chemoattractant, were increased in the airway lumen, suggesting a neutrophilic inflammatory reaction (41).

A recent study examined a large cohort of severe COPD patients during exacerbations and compared them to the stable state of the disease, but failed to demonstrate any change in sputum total cells and differential counts between exacerbated and stable disease. The only difference in the exacerbated group was a higher level of the proinflammatory cytokine IL-6 (42).

There are several possible explanations for the discrepancies observed among these studies, and they include the heterogeneity of the disease, the differences in baseline severity of patients, and the variable etiology of exacerbations.

REVERSIBILITY OF PATHOLOGIC LESIONS

The question whether smoking cessation or use of antiinflammatory drugs may reverse the airway inflammatory process present in COPD is still debated, and only a few studies performed a direct assessment of airway inflammation after smoking cessation or after corticosteroid therapy.

Although smoking is the principal cause of COPD, quitting smoking does not appear to result in resolution of the inflammatory response in the airways (43, 44). This suggests that there are perpetuating mechanisms that maintain the chronic inflammatory process once it has become established (38).

Corticosteroids are the most effective therapy for chronic asthma. The recognition that a chronic inflammatory process is also present in COPD provides a rationale for the use of antiinflammatory treatment, i.e. corticosteroids, in this disease. However, the type of inflammation in COPD is different from that in asthma and is not suppressed by inhaled or oral corticosteroids, even at high doses (45). A possible explanation for this lack of effect is the fact that corticosteroids prolong the survival of neutrophils by inhibition of neutrophil apoptosis (46). By contrast, these drugs are well effective against the prominent eosinophilia that characterizes airway inflammation in asthma.

Recent studies found that long term treatment with high doses of inhaled corticosteroids are unable to reduce the progression of COPD (47, 48), although they may reduce the incidence of acute exacerbations (49). A possible explanation for the effectiveness of corticosteroids in the exacerbations of COPD is the finding that the pattern of bronchial inflammation changes during an exacerbation of the disease, showing a prominent airway eosinophilia (39). The idea that eosinophilic inflammation is a marker for responsiveness to corticosteroids is supported by the recent observation that airway eosinophilia is present in a subgroup of patients with COPD who im-

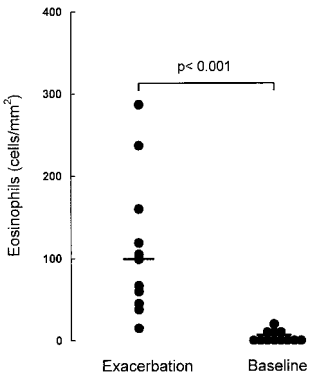

Figure 2. Individual values of eosinophils infiltrating the central airways of subjects with COPD examined in baseline conditions and during an exacerbation of the disease. From Reference 39.

prove their pulmonary function in response to a short course of steroids (50, 51).

These findings suggest the presence of a subgroup of patients with COPD characterized by "asthmatic features", such as airway eosinophilia and responsiveness to corticosteroids. It is possible that the majority of studies examining the effect of corticosteroids on COPD may have failed to obtain positive results because they excluded patients with "asthmatic features", therefore eliminating those who would have been most responsive to treatment.

OPEN QUESTIONS

Considering the increasing global prevalence of COPD and the consequent high health care costs, there is a need to better understand the mechanisms of this surprisingly neglected disease. In particular, the most relevant questions on the role of cellular and structural changes in COPD are:

1. Are the early stages of the disease still potentially reversible? The lack of tissue destruction and fibrosis observed in "normal" smokers would support this hypothesis.
2. Why do only a minority of smokers develop COPD? The fact that, for the same amount of cigarettes smoked, only some people are susceptible to COPD strongly suggests a genetic component or an environmental factor (i.e. infections, pollutants) triggering and/or maintaining the disease. The observations that people with respiratory infections in childhood are more prone to develop COPD and that smokers with established COPD have more CD8+ve cytotoxic T-lymphocytes in their lungs as compared with smokers with normal lung function would support the role of viral infections in the development of airflow limitation in susceptible smokers. Alternatively, it is also possible that an "autoimmune" mechanism, involving CD8+ve cytotoxic T-lymphocytes, could play a role.
3. Are mild COPD and severe COPD different diseases or different stages of the same disease? The observation that a prominent airway wall neutrophilia, that is not present in mild COPD, occurs in smokers with severe COPD does not help to solve the problem. In fact we still don't know whether this neutrophilia reflects a different pathology or simply represents a marker of severity of the disease. On the other hand the few autopsy data available seem to indicate that, in severe COPD, the predominant lesion would be the parenchymal destruction, whereas in mild COPD, the predominant lesion would be the inflammation of peripheral airways, thus suggesting that mild and severe COPD are two different diseases. However, since parenchymal destruction and peripheral airway inflammation often coexist in the same subject, it is also possible that both these pathologic lesions contribute to COPD of different severity, thus indicating that mild and severe COPD are different stages of the same disease.
4. Are COPD patients with frequent exacerbations more prone to have an accelerated decline in lung function? The burst of inflammatory cells, either eosinophils or neutrophils, occurring in the airways during an exacerbation of the disease, could potentially damage the lung, thus contributing to the progression of airflow limitation. On the other hand, it is also possible that these inflammatory cells simply represent an aspecific reaction that does not influence the progression of the disease.
5. Are the pathologic lesions characteristic of established COPD potentially reversible? It would seem reasonable to think that lesions such as parenchymal destruction and fibrosis are irreversible, whereas lesions such as airway inflammation could be potentially reversible, but further studies are needed to support this hypothesis. At the moment we have only a small piece of information, i.e. when airway inflammation is eosinophilic in nature, then a potential reversibility is present.

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