Pathology of COPD and Asthma

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3.1 Introduction

Although there is no doubt that the major site of increased resistance in COPD locates in the peripheral airways, the central airways are involved in the disease as well [1–5]. Conversely, in asthma, though the majority of studies focused on the pathophysiology of central airways, there is an increasing appreciation of a critical involvement of the peripheral airways [6]. Distinctive patterns of airway inflammation and structural remodelling are constitutive parts of the pathological picture of the two diseases [7, 8]. These include both cellular and structural changes that may contribute to the clinical manifestations and functional impairment characteristic of each condition.

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3.2 Conducting Airways

3.2.1 Asthma

Much of the current knowledge on the mechanisms of asthma came from studies performed on allergic asthma, where antigen exposure could activate antigen presenting cells and, in turn, T-lymphocytes, thus inducing an immune response characterised by a shift towards a Th2 phenotype, with production (among other cytokines) of IL-4, that sustains increased IgE levels, and of IL-5, that promotes eosinophilia. Accordingly, the characteristic airway inflammatory response involves activated T-lymphocytes, eosinophils and mast cells [7, 9–11]. In particular, mast cells infiltrating the airway muscle layer are believed to have important implications in asthma by affecting the degree of airway reactivity and airway remodelling [11]. Indeed, mast cells can release a variety of mediators, including those that induce airway smooth muscle contraction, resulting in bronchoconstriction, but also those that promote the development of structural changes in the airways, thus airway remodelling. The characteristic components of airway remodelling in asthma are: increased smooth muscle mass, thickening of the reticular basement membrane (RBM), angiogenesis and bronchial epithelial damage [7, 12].

Traditionally, remodelling has been considered the unavoidable consequence of long-term inflammation, but the exact relationship between airway inflammation and remodelling is still poorly understood. Of interest, the first studies that evaluated the pathology of asthma in children showed that both airway eosinophilia and all the structural changes characteristic of asthma were already present even in young children, at the first stages of the disease [13–15]. These observations indicate that the processes leading to remodelling of the airway wall begin early in the course of the disease and most probably occur in parallel with the establishment of chronic inflammation rather than being a consequence of it. Furthermore, recent evidence suggests that the presence of eosinophils and eosinophilic mediators is not required for the development of airway remodelling [16, 17]. Indeed, although eosinophil levels are on average increased in asthmatic patients, up to 50 % of asthmatic subjects do not show evidence of airway eosinophilia despite having all the clinical and functional features of the disease. Of interest, we have demonstrated that the typical aspects of airway remodelling (epithelial damage, basement membrane thickening and angiogenesis) develop in asthmatic children even in the absence of eosinophils or the eosinophil-related cytokines IL-4 and IL-5 [16]. In line with our observations, a recent study provided evidence that bronchoconstriction, independently of inflammation, may induce epithelial stress and initiate a tissue response that leads to structural changes in the airways [17]. These results do not throw into question the importance of eosinophils as effector cells in asthma, but rather suggest that other pathways may be involved in remodelling, thus highlighting the complexity of the disease. Finally, although eosinophils, T-lymphocytes and mast cells are the predominant cell types in asthma, evidence is now emerging that neutrophils may play a key role, at least in a subset of patients. Neutrophils have been traditionally associated with severe asthma [18–20], but subsequent studies showed that this phenotype is rather common even among patients with milder forms of the disease [21, 22]. Several factors can contribute to airway neutrophilia,

including respiratory infections, smoking and corticosteroid therapy, which reduces eosinophils but increases neutrophils inhibiting their apoptosis [22, 23].

As highlighted above, most of the studies on the pathogenesis of asthma performed so far traditionally concentrated on environmental stimuli, mainly aeroallergens, and the consequent adaptive immune response with priming of Th2 T-lymphocytes and recruitment of eosinophils. However, this is probably not the only pathway, since airway eosinophilia and remodelling are present not only in children with atopic asthma but even in those with non-atopic asthma, indicating that the airway pathology characteristic of asthma may develop even in the absence of atopy [24]. Of interest, it is becoming increasingly evident that disturbance of innate immune responses could play a key role in the pathogenesis of asthma which has been underappreciated so far. In this context viral infections, and particularly rhinoviruses, probably play a crucial role. Viral upper respiratory tract infections are, directly or indirectly, responsible for vast health-care use worldwide. Viral infections are particularly important in asthmatic patients, in whom rhinovirus is the most frequent cause of exacerbations both in adults and children [25]. Moreover, data from longitudinal studies suggest that wheezing episodes associated with viral infections early in life are a major risk factor for the development of asthma later in life [26, 27]. Impaired immune response to viral infections, with decreased IFN production, has been proposed as a mechanism for increased susceptibility to infections in asthmatic patients. Indeed, previous studies suggested that the immune response to viral infections is deficient in adult atopic asthmatics and that this deficiency correlates with the severity of virus-induced asthma exacerbations and asthma symptoms [28–30]. Whether this abnormal immune response is already present in the airways of young children or whether it develops later on as a consequence of long-term immune deregulation or sustained corticosteroid therapy was not known. To address this issue we have recently studied the epithelial production of IFN-β and IFN-λ in response to rhinovirus and reported a deficient IFN response in children with asthma, not only in those with atopy but also in non-atopic ones [31]. This altered innate response was associated to increased viral replication ex vivo and was correlated with the degree of airway eosinophilia and epithelial damage. These results suggest that disturbance of the airway immunopathological profile early in life, by affecting innate immune responses, could explain the greater susceptibility to viral infections observed in asthmatic patients and, possibly, the persistence of symptoms.

The role of the peripheral airways in asthma is increasingly being recognised as a relevant target for asthma knowledge and adequate control [32]. Evidence accumulating in recent decades indicates that inflammatory changes characteristic of the proximal airways of asthmatics also occur in the distal airways [33]. Of interest, the distribution of the inflammatory cell infiltration within the airway wall varies significantly moving from the central to the peripheral airways [34]. In central airways the preponderance of inflammatory cells has been observed in the airway submucosa (i.e. the inner area which lies between the epithelial basement membrane and the smooth muscle), whereas in peripheral airways the greatest density of eosinophils is in the adventitia (i.e. the external area which lies between the smooth muscle and the alveolar attachments). These regional differences in inflammatory cell density could have important physiologic implications in the relative mechanisms contributing to airflow limitation at these two anatomic sites. In large airways the

increased eosinophil density in the "inner" region would promote airway constriction by amplifying the effect of bronchial smooth muscle shortening on the airway calibre. Conversely, in peripheral airways the increased eosinophil density in the "outer" region would promote airway constriction by decreasing the tethering effects of the parenchyma on the airway wall [35].

3.2.2 COPD

The pathological mechanisms leading to airflow limitation in chronic obstructive pulmonary disease have been the focus of several studies that highlighted the concept, now well accepted, that COPD is also characterised by an important airway inflammatory process involving the central and the peripheral airways [4, 5, 36]. Cigarette smoking is the most important risk factor for the development of COPD, and it has long been recognised that smokers show evidence of inflammatory changes in their airways, consisting predominantly of macrophages infiltration in the airway wall and neutrophil accumulation in the airway lumen [2]. This early inflammatory infiltrate probably represents the non-specific response of the innate immunity to the insult of cigarette smoking. In smokers who develop chronic airflow limitation, this inflammatory process is further amplified, due to the activation of an adaptive immune response [37]. Indeed, in smokers who develop COPD, there is an increase in the number of lymphocytes (particularly $CD8^+$ T-lymphocytes and B-lymphocytes) and macrophages [4, 5, 36–39]. High numbers of $CD8^+$ T-lymphocytes are present not only in the peripheral airways of smokers with COPD but also in central airways in the lung parenchyma and in the adventitia of the pulmonary arterioles, suggesting that it is a consistent trait in this disease [4, 5, 40]. The findings of increased numbers of lymphocytes, and especially CD8+ T-cells, only in smokers who develop COPD is intriguing and supports the notion that a T-cell inflammation may be essential for the development of the disease. Traditionally the major activity of CD8 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 [41]. It is conceivable that, in response to repeated viral infections, an excessive recruitment of CD8 cytotoxic T-lymphocytes may occur and damage the lung in susceptible smokers, possibly through the release of perforins and TNF-α [42]. On the other hand, it is also possible that CD8 T-lymphocytes are able to damage the lung even in the absence of a stimulus such as a viral infection, as shown by Enelow and coworkers [43] who demonstrated that recognition of a lung "autoantigen" by cytotoxic T-cells may directly produce a marked lung injury. Along with the inflammatory response, several structural changes have been described in the conducting airways of smokers with established COPD that would result in narrowing of the airway lumen and in loss of the tethering function of the lung parenchyma, thus promoting a reduction of expiratory flow. These pathological lesions include thickening of the airway wall, with increased smooth muscle mass and fibrosis, hypertrophy of mucous glands and hyperplasia of goblet cells [36, 44, 45].

A significantly increased number of mucus-secreting goblet cells are seen in the peripheral airway epithelium of smokers with COPD. The increased number of goblet cells correlates with the degree of lung function impairment, as assessed by FEV_1/FVC [45]. This goblet cell metaplasia can have important functional consequences, potentially contributing to the development of smoking-induced airflow obstruction in at least two ways: first, by producing an excess of mucus which could alter the surface tension of the airway lining fluid, rendering the peripheral airways unstable and facilitating their closure and second, by inducing luminal occlusion through the formation of mucous plugs in peripheral airways. Indeed, luminal occlusion by mucous and inflammatory exudates is frequently observed in smokers with COPD [46, 47]. In addition neutrophils, which are not usually found within the airway wall, are increased in the peripheral airway epithelium and in bronchial glands of smokers with COPD [45, 48]. As neutrophil elastase is a remarkably potent secretagogue, it has been proposed that the location of neutrophils in close contact with the mucus-secreting structures of the glands and of the epithelium is crucial for the activation of their secretory function. While the excessive mucus production from goblet cells in peripheral airways may indeed contribute to airway obstruction, whether chronic bronchitis (due to mucus hypersecretion from bronchial glands in the central airways) could promote the development of functional abnormalities has been the matter of an extensive debate, with no definite answer yet. Nevertheless, it is now increasingly being recognised that, when present in patients with COPD, chronic bronchitis has numerous clinical consequences including an increased exacerbation rate, accelerated decline in lung function, worse quality of life and, possibly, increased mortality [49, 50].

Besides their role on mucus hypersecretion, neutrophils may have important effector functions even on airway smooth muscle. Indeed, an enlarged smooth muscle area is an important component of airway wall thickening, which is increased in smokers with COPD compared with those with normal lung function and augments progressively with worsening of airflow limitation [36]. This increase in smooth muscle can be due to several mechanisms, including hypertrophy and hyperplasia of smooth muscle cells and matrix deposition within smooth muscle bundles. Of interest, we reported an increased number of neutrophils infiltrating the smooth muscle of patients with COPD; these cells through the release of inflammatory mediators, cytokines and growth factors, could modulate smooth muscle proliferation and contractility [51]. Indeed, it is well known that the airways of smokers can react to non-specific stimuli by constricting, and this constriction results in airway hyperreactivity. Whether hyperresponsiveness is a primary event that might contribute to the natural history of COPD or is a consequence of the already decreased airway dimensions is still an open question. In any case, the abnormalities found in the airways of smokers, particularly chronic inflammation, could contribute to the constriction even of a normal airway smooth muscle.

Another important component of remodelling is fibrosis of the airway wall. It has been previously reported that cigarette smoke induces oxidative stress in human lung fibroblasts, which may then initiate a process of repair and collagen deposition [52]. Furthermore, the interaction between fibroblasts and inflammatory cells may

also play a role in fibrotic remodelling. On this line is the observation that mast cells, which have important profibrotic and prorepair properties, are increased in the airways of smokers with COPD, particularly in those with centrilobular emphysema [53]. Fibrosis, along with an increased airway smooth muscle and other inflammatory components, ought to increase the airway wall thickness and change the mechanical characteristics of the airway to decrease the luminal diameter. Indeed, the same degree of smooth muscle shortening may cause considerably greater luminal narrowing in airways with a thickened airway wall than in normal airways [54]. In the context of a chronic disease such as COPD, it is well conceivable that the pathological changes observed in small airways are associated to various attempts to repair, which may result in fibrosis, and thickening of the airway wall. On this line it should be noted that thickening of the airway wall is the parameter found to correlate best with airflow limitation in smokers across the different stages of disease severity [46, 55].

3.3 Lung Parenchyma

3.3.1 Asthma

Only a few studies have examined the inflammation of the lung parenchyma in asthma, focusing their analysis on transbronchial biopsies [20, 56, 57]. Patients with nocturnal asthma, when examined at night, have an increase in the number of alveolar tissue eosinophils and CD4 T-lymphocytes as compared with those with non-nocturnal asthma, and these cells correlate with the overnight decrement in lung function [56]. Furthermore, patients with uncontrolled asthma had increased number of mast cells in the alveolar walls, with increased expression of FcεRI and surface-bound IgE, compared to healthy controls [57]. Finally, in the alveolar walls of patients with severe steroid-dependent asthma, the inflammatory response is characterised by a prominent neutrophilia [22] which could represent a marker of severity, but could also be a consequence of corticosteroid therapy.

Taken together, these results point towards the presence of an excessive alveolar inflammation particularly in asthma phenotypes difficult to control. Moreover, they go along with the observation that inflammation in small airways predominates in the outer region of the airway wall that is in the adventitial layer and may spread out to the surrounding alveolar walls that is the site of alveolar attachments. Indeed, it is known that the elastic load provided by the lung parenchyma is transmitted to the airways through the alveolar attachments, resulting in mechanical interdependence between airways and parenchyma [54]. Of note, it has been shown that patients who died of fatal asthma have an increased number of damaged alveolar attachments and decreased elastic fibre content in the adventitial layer of small airways and peribronchial alveoli [58]. These alterations can contribute to the pathogenesis of some of the functional abnormalities observed in patients with the most severe forms of asthma, such as the loss of deep breath bronchodilator effect and enhanced airway closure [59].

3.3.2 COPD

Investigation of the inflammatory changes in the alveolar region of COPD patients is of particular interest, as a localisation of inflammatory cells within the alveolar walls might contribute to the smoking-induced parenchymal destruction that characterises the disease. Emphysema, which is one of the major contributors to airflow limitation in COPD, is defined anatomically as a permanent "destructive" enlargement of airspaces distal to the terminal bronchiole without obvious fibrosis [60]. However, this last statement has been debated since some studies have shown that, in emphysema, the destructive process is accompanied by a net increase in the mass of collagen, suggesting that, contrary to the definition of the disease, there is indeed an active alveolar wall fibrosis in emphysematous lungs [61, 62].

Smokers can develop two main morphological forms of emphysema that can be distinguished according to the region of the acinus which is destroyed. Centriacinar (or centrilobular) emphysema is characterised by focal destruction restricted to respiratory bronchioles 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 characterised 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 early in life, usually associated with deficiency of alpha1-antitrypsin, and in contrast to the centriacinar form has a tendency to involve the lower lobes more than the upper ones. Nonetheless, heavy smokers with normal alpha1-antitrypsin levels can develop both the centrilobular and panlobular phenotype [63].

The two forms of emphysema have distinct mechanical properties and distinct peripheral airway involvement [63, 64]. 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. Thus, in panlobular emphysema, airflow limitation seems to be primarily a function of loss of elastic recoil as suggested by the correlation between reduced expiratory flow and increased compliance observed in this form of emphysema [64]. By contrast, in centrilobular emphysema airflow limitation seems primarily a function of peripheral airway inflammation, as supported by the correlation between reduced expiratory flow and increased airway inflammation. In support of a central role of inflammation in this form of emphysema, we recently reported that patients with centrilobular emphysema show a more severe inflammatory infiltrate in both the lung parenchyma and peripheral airways. Mast-cell infiltration was a prominent component of this response and was related to the degree of airway reactivity, suggesting that centrilobular emphysema shares some pathogenetic traits with asthma [53].

Inflammation has been identified as a key component of COPD, and it has been shown that inflammatory cell infiltration in the lung can persist for years after cessation of smoking [65]. The implication of an inflammatory response to the pathogenesis of emphysema is not new, but our knowledge of the mechanisms regulating the activation and persistence of this response in the lung is continuously evolving.

Starting from the hypothesis of elastase-antielastase imbalance, proposed more than 40 years ago, studies first focused on the role of neutrophils and macrophages and their ability to induce lung destruction through the release of proteolytic enzymes. However, the activation of neutrophils and macrophages by itself is not sufficient to explain all the pathogenetic traits of COPD, and more recent evidences suggest a crucial role for acquired immunity, with involvement of dendritic cells and lymphocytes [37]. This hypothesis was based on the observation that B- and T-lymphocytes, especially of the $CD8^+$ subset, were the predominant cells infiltrating lung tissue of patients with COPD, and their numbers were strongly related to the apoptosis of structural cells and lung function impairment [36–40]. Adding to this, it has been recently proposed that this adaptive immune response, at least in some patients, could have an autoimmune component due to the recognition of pulmonary self-antigens modified by cigarette smoking and to the failure of mechanisms regulating immunological tolerance. In support of this hypothesis is the observation that, in non-smoking subjects who develop COPD, the disease seems to be associated with organ-specific autoimmunity [66].

3.4 Overlap between Asthma and COPD

As we have seen, asthma and COPD are two distinct obstructive lung diseases with distinctive clinical presentations and different patterns of airway inflammation and remodelling of lung structure. Indeed, the two conditions usually differ in the pattern of inflammatory cells most frequently encountered and the typical structural changes. Asthma is characterised by an increase of eosinophils, $CD4^+$ T-lymphocytes and mast cells; whereas, in COPD, $CD8^+$T-lymphocytes, macrophages and neutrophils predominate. Furthermore, a typical increase in basement membrane thickness is frequently observed in patients with asthma that is not present in those with COPD.

Nevertheless, despite the distinctive features of the two conditions, some asthmatic patients may experience a fixed airflow obstruction that persists despite optimal pharmacologic treatment [67]. Indeed, up to 30 % of subjects with airflow obstruction have a history of asthma rather than COPD, and these are patients usually excluded from clinical trials as they cannot be labelled as having either asthma or COPD. Instead, they would deserve clinical and research attention because their disease is usually misjudged, their prognosis is unknown, and treatment has never been properly explored. Even the pathological traits at bases of asthma with fixed airflow obstruction were not completely understood. In particular, it was unknown whether these patients will maintain the pathological changes typical of asthma or whether, with the development of irreversible airflow obstruction, they will show the features typical of COPD. Of interest, we have shown that, within a group of patients with fixed airflow obstruction, those with a history of asthma have a distinct airway pathology compared with those with a history of smoking-induced COPD. Indeed, patients with a history of asthma and fixed airflow obstruction have the same pathological changes that are present in patients with asthma with variable

airflow obstruction in terms of both eosinophilia and increased basement membrane thickness [68]. These findings suggest that the asthmatic airway pathology does not change with the development of fixed airflow obstruction and, thus, does not become similar to the one characteristic of COPD. Furthermore, in a longitudinal follow-up of that study, we have shown that patients with fixed airflow obstruction due to asthma, just as patients with COPD, have accelerated lung function decline and increased frequency of exacerbations compared with asthmatic patients fully reversible to bronchodilators [69]. Yet, the lung function decline in patients with fixed airflow obstruction is associated with the pathogenetic substrates specific for the underlying disease, i.e. asthma vs. COPD. In particular, the fall in FEV_1 correlates with exhaled nitric oxide levels and sputum eosinophils in asthmatic patients, while it correlates with neutrophil counts and emphysema score in patients with COPD [69]. In conclusion, while fixed airflow obstruction is associated with accelerated lung function decline both in asthma and in COPD, the rate of functional decline depends on the distinctive pathological and clinical features of the underlying diseases. These observations have important clinical implications since therapeutic strategies should be addressed to the different pathogenetic traits in the two diseases, and patients with fixed airflow obstruction due to asthma should not be grouped under the general heading of COPD.

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