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REVIEW

Chronic obstructive pulmonary disease: molecular and cellular mechanisms

P.J. Barnes*, S.D. Shapiro#, R.A. Pauwels¶

Chronic obstructive pulmonary disease: molecular and cellular mechanisms. P.J. Barnes, S.D. Shapiro, R.A. Pauwels. ©ERS Journals Ltd 2003.

ABSTRACT: Chronic obstructive pulmonary disease is a leading cause of death and disability, but has only recently been extensively explored from a cellular and molecular perspective.

There is a chronic inflammation that leads to fixed narrowing of small airways and alveolar wall destruction (emphysema). This is characterised by increased numbers of alveolar macrophages, neutrophils and cytotoxic T-lymphocytes, and the release of multiple inflammatory mediators (lipids, chemokines, cytokines, growth factors). A high level of oxidative stress may amplify this inflammation. There is also increased elastolysis and evidence for involvement of several elastolytic enzymes, including serine proteases, cathepsins and matrix metalloproteinases.

The inflammation and proteolysis in chronic obstructive pulmonary disease is an amplification of the normal inflammatory response to cigarette smoke. This inflammation, in marked contrast to asthma, appears to be resistant to corticosteroids, prompting a search for novel anti-inflammatory therapies that may prevent the relentless progression of the disease.

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*National Heart and Lung Institute, Imperial College, London, UK, #Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA, ¶Dept of Respiratory Diseases, Ghent University Hospital, Ghent, Belgium.

Correspondence: P.J. Barnes, National Heart and Lung Institute, Imperial College School of Medicine, Dovehouse St, London SW3 6LY, UK
Fax: 44 2073515675
E-mail: p.j.barnes@imperial.ac.uk

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This review is a summary of a meeting on "COPD: the important questions" held in Malta in November 2002. The aim of the meeting was to identify important questions related to the cellular and molecular mechanism involved in chronic obstructive pulmonary disease (COPD) and to discuss new research approaches to gain a better understanding of the basic mechanisms involved in COPD.

COPD is a major and increasing global health problem, which is predicted to become the third commonest cause of death and the fifth commonest cause of disability in the world by 2020 [1]. While there have been major advances in the understanding and management of asthma, COPD has been relatively neglected and there are no current therapies that reduce the inevitable progression of this disease. However, because of the enormous burden of disease and escalating healthcare costs, there is now renewed interest in the underlying cellular and molecular mechanisms [2] and a search for new therapies [3], resulting in a re-evaluation of the disease [4]. Despite its enormous global importance, there has been relatively little research into COPD and it is the most underfunded disease in relation to the global burden of disease [5].

COPD is characterised by slowly progressive development of airflow limitation that is poorly reversible, in sharp contrast to asthma where there is variable airflow obstruction that is usually reversible spontaneously or with treatment. A new definition of COPD has recently been adopted by the Global Initiative on Obstructive Lung Disease (GOLD): "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases" [6]. For the first time this definition encompasses the idea that COPD is a chronic inflammatory disease and much of the recent research has focused on the nature of this inflammatory response.

COPD includes chronic obstructive bronchiolitis with fibrosis and obstruction of small airways, and emphysema with enlargement of airspaces and destruction of lung parenchyma, loss of lung elasticity and closure of small airways. Chronic bronchitis, by contrast, is defined by a productive cough of >3 months duration for more than two successive years; this reflects mucous hypersecretion and is not necessarily associated with airflow limitation. Most patients with COPD have all three pathological mechanisms (chronic obstructive bronchiolitis, emphysema and mucus plugging) as all are induced by smoking, but may differ in the proportion of emphysema and obstructive bronchiolitis [2]. In developed countries cigarette smoking is by far the commonest cause of COPD accounting for >95% of cases, but there are several other risk factors, including air pollution (particularly indoor air pollution from burning fuels), poor diet, and occupational exposure. COPD is characterised by acceleration in the normal decline of lung function seen with age. The slowly progressive airflow limitation leads to disability and premature death and is quite different from the variable

airway obstruction and symptoms in asthma, which rarely progress in severity. While COPD and asthma both involve inflammation in the respiratory tract there are marked differences in the nature of the inflammatory process, with differences in inflammatory cells, mediators, response to inflammation, anatomical distribution and response to anti-inflammatory therapy [4, 7]. Some patients appear to share the characteristics of COPD and asthma, however. Rather than this representing a graded spectrum of disease, it is more likely that these patients have both of these common diseases at the same time.

Differences from asthma

Histopathological studies of COPD show a predominant involvement of peripheral airways (bronchioles) and lung parenchyma, whereas asthma involves inflammation in all airways but usually without involvement of the lung parenchyma [8]. There is obstruction of bronchioles, with fibrosis and infiltration with macrophages and T-lymphocytes. There is destruction of lung parenchyma and an increased number of macrophages and T-lymphocytes, with a greater increase in $CD8^+$ (cytotoxic) than $CD4^+$ (helper) cells [9]. Bronchial biopsies show similar changes with an infiltration of macrophages and $CD8^+$ cells and an increased number of neutrophils in patients with severe COPD [10]. Bronchoalveolar lavage (BAL) fluid and induced sputum demonstrate a marked increase in macrophages and neutrophils [11, 12]. In contrast to asthma, eosinophils are not prominent except during exacerbations or when patients have concomitant asthma [8, 13].

What are the predominant mechanisms of airflow limitation?

Fixed narrowing of small airways, emphysema and luminal obstruction with mucus secretions may all contribute to airflow limitation in COPD, but there is debate about which mechanism is most important. There are differences between patients and at different stages of disease progression in the contribution of each of these processes but problems in making accurate measurements in patients has made it difficult to evaluate the importance of each mechanism in an individual patient.

Small airways

It has long been recognised that there is narrowing of small airways in patients with COPD [14–17]. There is an increase in the thickness of small airways with increased formation of lymphoid follicles and deposition of collagen in the outer airway wall that may restrict airway opening [18]. The lumen of small airways is reduced by mucosal thickening containing an inflammatory exudate, which increases with the severity of disease. The mechanism for lymphoid follicle formation in more severe disease is unknown, but may reflect a response to chronic bacterial colonisation and acute exacerbations of inflammation. The mechanisms of fibrosis around the airway are not yet understood, but are likely to represent an attempt to repair chronic inflammation. The role of specific growth factors, such as transforming growth factor-β (TGF-β) which shows increased expression in peripheral airways [19, 20] and connective tissue growth factor (CTGF) are not yet known. TGF-β may induce fibrosis *via* the release of CTGF which may stimulate collagen deposition in the airways [21, 22]. A major barrier to understanding the contribution of small airway obstruction is the difficulty in quantifying small airway obstruction in patients using measurements of airflow due to the high variability and poor reproducibility of measurements [23].

Emphysema

Both panacinar and centrilobular emphysema may occur in smokers [24]. The role of emphysema in causing airflow obstruction in COPD have been examined by measuring macroscopic emphysema in resected lung or on computerised tomography (CT) scans in relation to tests of lung function, or by measuring static transpulmonary pressure (P_L) as a measurement of alveolar disease. Many studies have shown significant, albeit weak, correlations between the grading of macroscopic emphysema and various tests of lung function [25, 26]. However, the assessment of macroscopic emphysema is dominated by destroyed or poorly functioning lung, whereas lung function tests reflect predominantly the function of the best surviving lung. In the extreme case of nonventilated emphysematous bullae surrounded by normal lung, the two assessments are virtually independent, with lung function tests measuring only the reduced volume of surviving lung. In more common types of emphysema, a simple two compartment model does not apply, but usually there will be greater heterogeneity of disease with more and more units becoming poorly functional as disease progresses, resulting in a rise in residual volume and a fall in vital capacity. Thus the strength of the correlation between assessment of gross emphysema and lung function will depend on the severity and homogeneity of "microscopic" disease in the less affected lung which is not often measured.

P_L (measured using an oesophageal catheter) is plotted against airflow conductance or maximal expiratory flow at different lung volumes to indicate the contribution of alveolar disease (and by implication emphysema) to airflow limitation, with the assumption that the rest is due to intrinsic airway disease [27–29]. However, it is not certain that the magnitude of decline in P_L accurately reflects the severity of emphysema and its effects on the airways. Reduction in P_L is likely to be largest with relatively uniform emphysema, as occurs in panacinar emphysema (*e.g.* α_1-antitrypsin deficiency), whereas patchy centrilobular emphysema may have near normal P_L. Indeed, uniform "microscopic" emphysema might account for functional "pseudoemphysema" without any CT change.

In practice a reduction in conductance or maximum flow that is completely explained by a reduction in P_L is unusual, except in mild disease. Retrograde catheter studies in excised lungs from patients with severe airflow obstruction due to COPD have all found large increases in peripheral resistance at standard P_L [30–32]. But there are many other possible changes in airway function due to emphysema which would result in an increased resistance at a given P_L, including abnormal angulation or compression of normal airways by surrounding overdistended lung, loss of parallel airways due to emphysematous destruction, or to functional loss of patent airways supplying poorly ventilated areas of lung. The effects of emphysema may not always reduce P_L, *e.g.* a short stenosis caused by local loss of alveolar attachments. Present analyses of airway morphology are not sufficient to reveal the anatomical basis of the consistent physiological finding of an increase in peripheral airflow resistance. Assuming that the increase is all due to "intrinsic" disease of the peripheral airways underestimates the role of emphysema. Emphysema may play a more prominent role in severe disease as the decline in lung function accelerates.

Mucus hypersecretion

The contribution of mucus hypersecretion to airflow limitation in COPD is still uncertain. Although early studies supported the view that mucus hypersecretion was not associated with any physiological defect [33, 34], more recent studies have demonstrated that mucus hypersecretion may be a potential risk factor for accelerated decline in lung function [35, 36]. The early studies examined the early stages of COPD and also included an occupational cohort. The most likely mechanism whereby chronic mucus hypersecretion contributes to progression of COPD may be due to the increased risk of exacerbations that appear to accelerate loss of forced expiratory volume in one second (FEV_1) [37]. Chronic mucus hypersecretion may contribute little in the early phases of COPD when exacerbations are infrequent. It is possible that chronic mucus hypersecretion may reflect the inflammatory process around submucosal glands [38] and may reflect the intensity of inflammation in more peripheral airways. Increased numbers of neutrophils and mast cells have also been found around submucosal glands [38, 39] and serine proteases and mast cell chymase are potent mucus secretagogues [40–42]. In severe COPD chronic mucus hypersecretion is associated with mortality and this may also reflect an increased risk of terminal infection [43–45]. Chronic cough and mucus production in smokers with normal lung function (GOLD Stage 0) do not appear to predict the later development of COPD [46].

What are the key inflammatory cells?

COPD, like asthma, is a complex inflammatory disease that involves several types of inflammatory cells and multiple inflammatory mediators. However, the pattern of inflammation and the spectrum of mediators differ between these two airway diseases, at least in the stable state of the disease. Although abnormal numbers of inflammatory cells have been documented in COPD, the relationship between these cell types and the sequence of their appearance and their persistence are largely unknown. Most studies have been cross-sectional based on selection of patients with different stages of the disease and comparisons have been made between smokers without airflow limitation (normal smokers) and those with COPD who have smoked a similar amount. There are no serial studies and selection biases (such as selecting tissue from patients suitable for lung volume reduction surgery) may give misleading results. Analysis of the cell profile in alveoli and small airways shows an increase in all of the cell types implicated in COPD, including macrophages, T-lymphocytes, B-lymphocytes and neutrophils [47].

Neutrophils

Increased numbers of activated neutrophils are found in sputum and BAL fluid of patients with COPD [12, 48], yet there are only relatively small increases in the airways or lung parenchyma [49]. This may reflect their rapid transit through the airways and parenchyma. Neutrophils secrete serine proteases, including neutrophil elastase (NE), cathepsin G and proteinase-3, as well as matrix metalloproteinase (MMP)-8 and MMP-9, which may contribute to alveolar destruction. These serine proteases are also potent mucus stimulants. Neutrophil recruitment to the airways and parenchyma involves adhesion to endothelial cells and E-selectin is upregulated on endothelial cells in the airways of COPD patients [50]. Adherent neutrophils then migrate into the respiratory tract under the direction of neutrophil chemotactic factors, which include interleukin (IL)-8 and leukotriene B_4 (LTB_4). Neutrophil survival in the respiratory tract may be increased by cytokines, such as granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF).

The role of neutrophils in COPD is not yet clear. There is a correlation between the number of circulating neutrophils and fall in FEV_1 [51]. Neutrophil numbers in bronchial biopsies and induced sputum are correlated with COPD disease severity [10, 12] and with the rate of decline in lung function [52]. Smoking has a direct stimulatory effect on granulocyte production and release from the bone marrow, possibly mediated by GM-CSF and G-CSF released from lung macrophages [53]. Smoking may also increases neutrophil retention in the lung [54]. There is no doubt that the neutrophils recruited to the airways of COPD patients are activated as there are increased concentrations of granule proteins, such as myeloperoxidase and human neutrophil lipocalin, in the sputum supernatant [55–57]. These neutrophils also show an increase in the respiratory burst response which correlates with the degree of airflow limitation [58].

Neutrophils have the capacity to induce tissue damage through the release of serine proteases and oxidants. Priming is a prerequisite for degranulation and superoxide anion generation in neutrophils [59]. Neutrophils in the peripheral circulation show evidence of priming in COPD [60], but this may result from, rather than contribute to, lung pathophysiology. There are several chemotactic signals that have the potential for neutrophil recruitment in COPD, including LTB_4, IL-8 and related CXC chemokines, including GRO-α (growth-related oncoprotein) and ENA-78 (epithelial neutrophil activating protein of 78 kDa) which are increased in COPD airways [61, 62]. These mediators may be derived from alveolar macrophages and epithelial cells, but the neutrophil itself may be a major source of IL-8 [63].

Neutrophils from the circulation marginate in the pulmonary circulation and adhere to endothelial cells in the alveolar wall before passing into the alveolar space [64]. The precise route for neutrophil migration in large airways is less certain, but it is more likely that they reach the airway from the tracheobronchial circulation and migrate across post-capillary venules [65]. The cellular mechanisms underlying neutrophil adhesion and transmigration differ between systemic and pulmonary circulations and this might confer different properties on the neutrophils arriving from the alveolar or bronchial compartments. There may be significant differences in neutrophil transit times in different areas of the lung that may account for differential distribution of emphysema, for example the upper lobe predominance in centrilobular emphysema. Little is known about survival and apoptosis of neutrophils in COPD airways. Theoretically GM-CSF may prolong neutrophil survival but it has proved difficult to culture neutrophils from sputum samples.

However, while neutrophils have the capacity to cause elastolysis, this is not a prominent feature of other pulmonary diseases where chronic airway neutrophilia is even more prominent, including cystic fibrosis and bronchiectasis. This suggests that other factors are involved in the generation of emphysema. Indeed there is a negative association between the number of neutrophils and the amount of alveolar destruction in COPD [49] and neutrophils are not a prominent feature of parenchymal inflammation in COPD. It is likely that airway neutrophilia is linked to mucus hypersecretion in chronic bronchitis, however. Serine proteases from neutrophils, including neutrophil elastase, cathepsin G and proteinase-3 are all potent stimulants of mucus secretion from submucosal glands and goblet cells in the epithelium [40, 42].

Macrophages

Macrophages appear to play a pivotal role in the pathophysiology of COPD and can account for most of the known features of the disease (fig. 1) [66, 67].

There is a marked increase (5–10 fold) in the numbers of macrophages in airways, lung parenchyma, BAL fluid and sputum in patients with COPD. A careful morphometric analysis of macrophage numbers in the parenchyma of patients with emphysema showed a 25-fold increase in the numbers of macrophages in the tissue and alveolar space compared with normal smokers [47]. Furthermore, macrophages are localised to sites of alveolar wall destruction in patients with emphysema [49, 68]. There is a correlation between macrophage numbers in the airways and the severity of COPD [10].

Macrophages may be activated by cigarette smoke extract to release inflammatory mediators, including tumour necrosis factor (TNF)-α, IL-8, other CXC chemokines, monocyte chemotactic peptide (MCP)-1, LTB_4 and reactive oxygen species, providing a cellular mechanism that links smoking with inflammation in COPD. Alveolar macrophages also secrete elastolytic enzymes, including MMP-2, MMP-9, MMP-12, cathepsins K, L and S and neutrophil elastase taken up from neutrophils [69, 70]. Alveolar macrophages from patients with COPD secrete more inflammatory proteins and have a greater elastolytic activity at baseline than those from normal smokers and this is further increased by exposure to cigarette smoke [70–72]. Macrophages demonstrate this difference even when maintained in culture for 3 days and therefore appear to be intrinsically different from the macrophages of normal smokers and nonsmoking normal control subjects [70]. The predominant elastolytic enzyme secreted by alveolar macrophages in COPD patients is MMP-9. Most of the inflammatory proteins that are upregulated in COPD macrophages are regulated by the transcription factor nuclear factor-κB (NF-κB) which is activated in alveolar macrophages of COPD patients, particularly during exacerbations [73, 74].

The increased numbers of macrophages in smokers and COPD patients may be due to increased recruitment of monocytes from the circulation in response to monocyte-selective chemokines. The monocyte-selective chemokine MCP-1 is increased in sputum and BAL of patients with COPD [61, 75], with increased expression in macrophages [76]. CXC chemokines are also chemoattractant to monocytes acting *via* CXCR2 and the concentration of the CXC chemokine GRO-α is markedly increased in sputum and BAL of patients with COPD [61]. Monocytes from patients with COPD show a greater chemotactic response to GRO-α than cells from normal smokers and nonsmokers, but this is not explained by an increase in CXCR2 [77]. Interestingly, while all monocytes express CCR2, the receptor for macrophage inflammatory protein-1, only ~30% of monocytes express CXCR2. It is possible that these CXCR2-expressing monocytes transform into macrophages that behave differently, *e.g.* release more inflammatory proteins. Macrophages also have the capacity to release the chemokines interferon-γ inducible protein (IP-10), interferon-inducible T-cell α-chemoattractant (I-TAC) and monokine-induced by interferon-γ (Mig), which are chemotactic for $CD8^+$ Tc1 cells *via* interaction with the CXCR3 receptor expressed on these cells [78].

The increased numbers of macrophages in COPD may be due to increased recruitment of monocytes, but may also be due to increased proliferation and prolonged survival in the lungs. Macrophages have a very low proliferation rate in the lungs, but the present authors' have demonstrated that there is some increase in cell proliferation measured by proliferative cell nuclear antigen (PCNA) [79]. Macrophages have a long survival time so this is difficult to measure directly. However in macrophages from smokers, there is markedly increased expression of the anti-apoptotic protein Bcl-X_L and increased expression of $p21^{CIP/WAF1}$ in the cytoplasm [79]. This suggests that macrophages may have a prolonged survival in smokers and patients with COPD.

Corticosteroids are ineffective in suppressing inflammation, including cytokines, chemokines and proteases, in patients

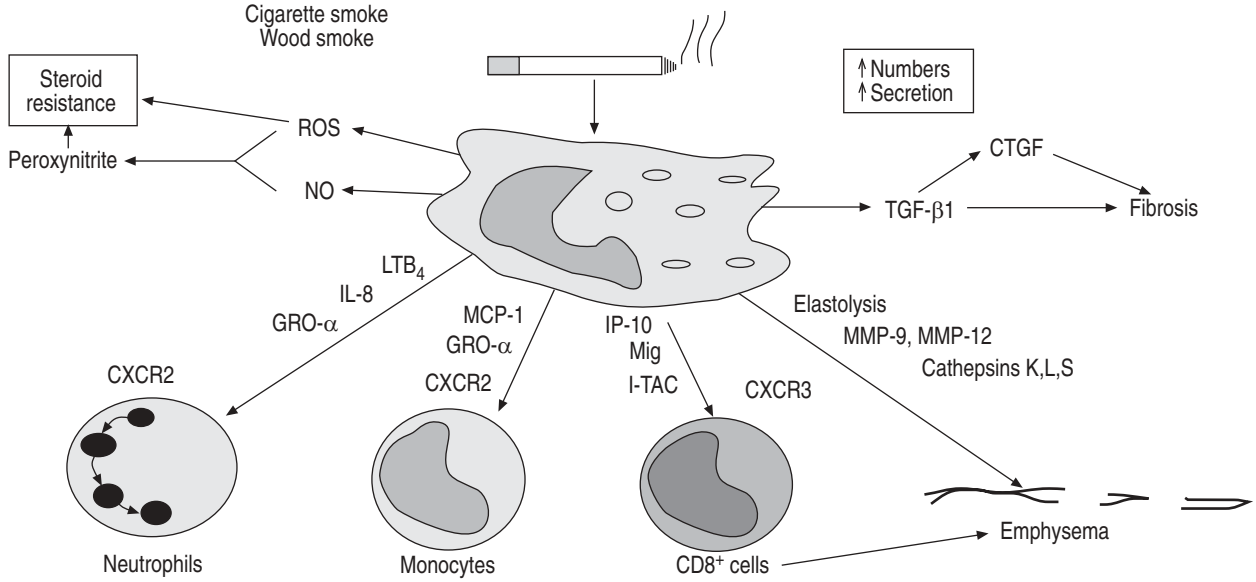

Fig. 1. – Macrophages may play a pivotal role in chronic obstructive pulmonary disease (COPD) as they are activated by cigarette smoke extract and secrete many inflammatory proteins that may orchestrate the inflammatory process in COPD. Neutrophils may be attracted by interleukin (IL)-8, growth-related oncogene-α (GRO-α) and leukotriene B_4 (LTB_4), monocytes by macrophage chemotactic protein-1 (MCP-1), and $CD8^+$ lymphocytes by interferon-γ inducible protein (IP-10), monokine-induced by interferon-γ (Mig) and interferon-inducible T-cell α-chemoattractant (I-TAC). Release of elastolytic enzymes including matrix metalloproteinases (MMP) and cathepsins cause elastolysis, and release of transforming growth factor (TGF-β) and connective tissue growth factor (CTGF). Macrophages also generate reactive oxygen species (ROS) and nitric oxide (NO) which together form peroxynitrite and may contribute to steroid resistance.

with COPD [80, 81]. *In vitro* the release of IL-8, TNF-α and MMP-9 macrophages from normal subjects and normal smokers are inhibited by corticosteroids, whereas corticosteroids are ineffective in macrophages from patients with COPD [82]. Curiously, this does not apply to GM-CSF which does not appear to have increased secretion in COPD and is suppressed by corticosteroids, albeit to a lesser extent than in macrophages from normal smokers. The reasons for resistance to corticosteroids in COPD and to a lesser extent macrophages from smokers may be the marked reduction in activity of histone deacetylase (HDAC) [83], which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes [84]. The reduction in HDAC activity in macrophages is correlated with increased secretion of cytokines like TNF-α and IL-8 and reduced response to corticosteroids. The reduction of HDAC activity on COPD patients may be mediated through oxidative stress and peroxynitrite formation.

Although corticosteroids are not effective in inhibiting the secretion of cytokines and proteases from macrophages, other drugs may be beneficial. Theophylline in low concentrations increases HDAC activity in alveolar macrophages and *in vitro* reverses the steroid resistance induced by oxidative stress [85]. Resveratrol, a flavenoid found in red wine, is an effective inhibitor of cytokine expression from macrophages from COPD patients, but its molecular mechanisms of action have not yet been determined [86].

Macrophages are phagocytic for bacteria and play an important role in host defence. The phagocytic potential of macrophages from COPD patients has not been explored, but it is possible that impaired phagocytosis may result in the increased bacterial load in the respiratory tract of patients with COPD. Macrophages recognise apoptotic cells *via* expression of phosphatidylserine (PS) which interacts with specific receptors on the macrophage surface [87]. Ingestion of apoptotic granulocytes by macrophages induces the secretion of TGF-β1 [88]. Neutrophil elastase cleaves the PS receptor and may thus impair the ability of macrophages to take up apoptotic neutrophils, resulting in increased numbers of apoptotic neutrophils in the airways [89].

T-lymphocytes

There is an increase in the total numbers of T-lymphocytes in lung parenchyma, peripheral and central airways of patients with COPD, with the greater increase in $CD8^+$ than $CD4^+$ cells [47, 49, 90–92]. There is a correlation between the numbers of T-cells and the amount of alveolar destruction and the severity of airflow obstruction. There is also an increase in the absolute number of $CD4^+$ T-cells, but the ratio of $CD4^+$:$CD8^+$ cells is reversed in COPD. This is mainly found in smokers with COPD rather than smokers without evidence of airflow limitation [90]. It is not known whether these cells are classified as Tc1 (interferon-γ producing) or Tc2 (IL-4 producing) subtypes [93], but there is evidence that the majority of T-cells in COPD airways are of the Tc1 subtype [78]. $CD8^+$ and $CD4^+$ T-cells show increased expression of activation markers compared to T-cells in the circulation, although there is no clear difference between patients with COPD and normal controls [94].

The mechanisms by which $CD8^+$, and to a lesser extent $CD4^+$ cells, accumulate in the airways and lungs of patients with COPD is not yet understood. However, homing of T-cells to the lung must depend upon some initial activation then adhesion and selective chemotaxis. T-cells in peripheral airways of COPD patients show increased expression of CXCR3, a receptor activated by IP-10, Mig and I-TAC. There is increased expression of IP-10 by bronchiolar epithelial cells and this could contribute to the accumulation of $CD8^+$ cells, which preferentially express CXCR3 [78].

There is also an increase in the numbers of $CD8^+$ cells in the circulation in COPD patients who do not smoke [95, 96] and an increase in T-helper type 1 (interferon (IFN)-γ producing) $CD4^+$ cells in COPD patients [97]. This indicates that there may be chronic immune stimulation *via* antigens presented *via* the HLA Class 1 pathway. Dendritic cells may migrate from the airways to regional lymph nodes and stimulate proliferation of $CD8^+$ and $CD4^+$ T-cells. $CD8^+$ cells are typically increased in airway infections and it is possible that the chronic colonisation of the lower respiratory tract of COPD patients by bacterial and viral pathogens is responsible for this inflammatory response [98]. It is also possible that protease-induced lung injury may uncover previously sequestered autoantigens or that cigarette smoke itself may damage airway epithelial cells and make them antigenic [99].

The role of increased numbers of $CD4^+$ cells in COPD, particularly in severe disease is also unknown [47]; it is possible that they have immunological memory and play a role in perpetuating the inflammatory process in the absence of cigarette smoking. Natural killer (NK, $CD56^+$) cells are the first line of defence against viral infections. Circulating NK cells are reduced in patients with COPD and have reduced phagocytic activity [100] and similar findings are found in normal smokers [101], although no difference in NK cells was found in lung parenchyma of COPD patients [90]. There is an increase in γ/δ T-cells in alveoli of smokers, whether they have airway obstruction or not [90].

The role of T-cells in the pathophysiology of COPD is not yet certain. $CD8^+$ cells have the capacity to cause cytolysis and apoptosis of alveolar epithelial cells through release of perforins, granzyme-B and TNF-α [102]. There is an association between $CD8^+$ cells and apoptosis of alveolar cells in emphysema [90]. In a mouse model of cigarette-induced emphysema there is a predominance of T-cells which are directly related to the severity of emphysema [103].

Eosinophils

The role of eosinophils in COPD is uncertain. There are some reports of increased numbers of inactive eosinophils in the airways and lavage of patients with stable COPD, whereas others have not found increased numbers in airway biopsies, BAL or induced sputum [104]. The presence of eosinophils in patients with COPD predicts a response to corticosteroids and may indicate coexisting asthma [105, 106]. Increased numbers of eosinophils have been reported in bronchial biopsies and BAL fluid during acute exacerbations of chronic bronchitis [107, 108]. Surprisingly the levels of eosinophil basic proteins in induced sputum are as elevated in COPD, as in asthma, despite the absence of eosinophils, suggesting that they may have degranulated and are no longer recognisable by microscopy [55]. Perhaps this is due to the high levels of neutrophil elastase that have been shown to cause degranulation of eosinophils [109].

Dendritic cells

The dendritic cell plays a central role in the initiation of the innate and adaptive immune response [110]. The airways and lungs contain a rich network of dendritic cells that are localised near the surface, so that they are ideally located to signal the entry of foreign substances that are inhaled [111]. Dendritic cells can activate a variety of other inflammatory

and immune cells, including macrophages, neutrophils, T- and B-lymphocytes [112]. It therefore likely that the dendritic cell may play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents and may therefore be a key cellular element in COPD (fig. 2). The mechanism by which tobacco smoke activates the immune system is not yet inderstood, but a glycoprotein isolated from tobacco has powerful immunostimulatory actions [113]. There is an increase in the number of dendritic cells in rat lungs exposed to cigarette smoke [114] and in the airways and alveolar walls of smokers [115, 116]. Pulmonary histiocytosis is a disease caused by dendritic cell granulomata in the lung and is characterised by destruction of the lung parenchyma that resembles emphysema [117, 118]. The adult form of the disease occurs almost exclusively in smokers. In mice exposed to chronic cigarette smoke there is an increase in dendritic cells in the airways and lung parenchyma [119]. The role of dendritic cells in recruiting other effector cells in COPD deserves further study.

Epithelial cells

Airway and alveolar epithelial cells may be an important source of inflammatory mediators and proteases in COPD. Epithelial cells are activated by cigarette smoke to produce inflammatory mediators, including TNF-α, IL-1β, GM-CSF and IL-8 [120–122]. Epithelial cells in small airways may be an important source of TGF-β, which then induces local fibrosis [20]. Vascular endothelial growth factor (VEGF) appears to be necessary to maintain alveolar cell survival and blockade of VEGF receptors (VEGFR2) in rats induces apoptosis of alveolar cells and an emphysema-like pathology [123]. However, the role of VEGF in the pathogenesis of human emphysema is not yet known.

Airway epithelial cells are also important in defence of the airways. Mucus produced from goblet cells traps bacteria and inhaled particulates [124]. Epithelial cells secrete defensins and other cationic peptides with antimicrobial effects and are part of the innate defence system, but are also involved in tissue repair processes [125]. They also secrete antioxidants as well as antiproteases, such as secretory leukoprotease inhibitor (SLPI). Epithelial cells also transport immunoglobulin A and are therefore also involved in adaptive immunity [126]. It is possible that cigarette smoke and other noxious agents impair these innate and adaptive immune responses of the airway epithelium, increasing susceptibility to infection.

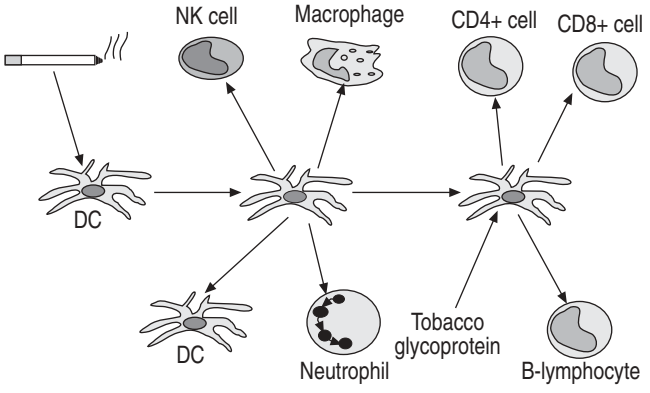

Fig. 2. – Dendritic cells (DC) may play an important role in the pathophysiology of chronic obstructive pulmonary disease as they can be activated by cigarettte smoking and by tobacco glycoprotein resulting in recruitment of neutrophils, macrophages, natural killer (NK) cells, $CD4^+$ and $CD8^+$ T-lymphocytes and B-lymphocytes.

The airway epithelium in chronic bronchitis and COPD often shows squamous metaplasia, which may result from increased proliferation of airway epithelial cells. Proliferation in basal airway epithelial cells, measured by PCNA is increased in some normal smokers, but is markedly increased in patients with chronic bronchitis and correlates with the degree of squamous metaplasia [127]. The nature of the growth factors involved in epithelial cell proliferation, cell cycle and differentiation in COPD are not yet known. Epithelial growth factor receptors show increased expression in airway epithelial cells of smokers and may contribute to basal cell proliferation, resulting in squamous metaplasia and an increased risk of bronchial carcinoma [128].

What is the role of oxidative stress?

Oxidative stress occurs when reactive oxygen species (ROS) are produced in excess of the antioxidant defence mechanisms and result in harmful effects, including damage to lipids, proteins and deoxyribonucleic acid (DNA). There is increasing evidence that oxidative stress is an important feature in COPD [129–131].

Formation

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including, neutrophils, eosinophils, macrophages, and epithelial cells [130]. Superoxide anions ($O_2^{\cdot-}$) are generated by reduced nicotinamine adenine dinucleotide phosphate (NADPH) oxidase and this is converted to hydrogen peroxide (H_2O_2) by superoxide dismutases. H_2O_2 is then dismuted to water by catalase. $O_2^{\cdot-}$ and H_2O_2 may interact in the presence of free iron to form the highly reactive hydroxyl radical ($^{\cdot}OH$). $O_2^{\cdot-}$ may also combine with NO to form peroxynitrite, which also generates $^{\cdot}OH$ [132]. Oxidative stress leads to the oxidation of arachidonic acid and the formation of a new series of prostanoid mediators called isoprostanes, which may exert significant functional effects [133], including bronchoconstriction and plasma exudation [134–136].

Granulocyte peroxidases, such as myeloperoxidase in neutrophils, play an important role in oxidative stress. In neutrophils H_2O_2 generated from O_2^- is metabolised by myeloperoxidase in the presence of chloride ions to hypochlorous acid which is a strong oxidant. Myeloperoxidase is also able to nitrate tyrosine residues, as can peroxynitrite [137–139].

Antioxidants

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract [140]. The major intracellular antioxidants in the airways are catalase, superoxide dismutase (SOD) and glutathione, formed by the enzyme γ-glutamyl cysteine synthetase, and glutathione synthetase. Oxidant stress activates the inducible enzyme haem oxygenase-1 (HO-1), converting haem and haemin to biliverdin with the formation of carbon monoxide (CO) [141]. Biliverdin is converted *via* bilirubin reductase to bilirubin, which is a potential antioxidant. HO-1 is widely expressed in human airways [142] and CO production is increased in COPD [143]. In the lung intracellular antioxidants are expressed at relatively low levels and are not induced by oxidative stress, whereas the major antioxidants are extracellular [144]. Extracellular antioxidants, particularly

glutathione peroxidase, are markedly upregulated in response to cigarette smoke and oxidative stress. The glutathione system is the major antioxidant mechanism in the airways. There is a high concentration of reduced glutathione in lung epithelial lining fluid [140] and concentrations are increased in cigarette smokers. Extracellular glutathione peroxidase (eGPx) is an important antioxidant in the lungs and may be secreted by epithelial cells and macrophages, particularly in response to cigarette smoke or oxidative stress [145]. eGPx inactivates H_2O_2 and O_2^-, but may also block reactive nitrogen species [144]. Extracellular antioxidants also include the dietary antioxidants vitamin C (ascorbic acid) and vitamin E (α-tocopherol), uric acid, lactoferrin and extracellular SOD3. SOD3 is highly expressed in human lung, but its role in COPD is not yet clear [146].

Effects on airways

ROS have several effects on the airways, which would have the effect of increasing the inflammatory response. These effects may be mediated by direct actions of ROS on target cells in the airways, but may also be mediated indirectly *via* activation of signal transduction pathways and transcription factors and *via* the formation of oxidised mediators such as isoprostanes and hydroxyl-nonenal.

ROS activate NF-κB, which switches on multiple inflammatory genes resulting in amplification of the inflammatory response [147]. The molecular pathways by which oxidative stress activates NF-κB have not been fully elucidated, but there are several redox-sensitive steps in the activation pathway [148]. Many of the stimuli that activate NF-κB appear to do so *via* the formation of ROS, particularly H_2O_2. ROS activate NF-κB in an epithelial cell line [149] and increase the release of pro-inflammatory cytokines from cultured human airway epithelial cells [150]. Oxidative stress results in activation of histone acetyltransferase activity which opens up the chromatin structure and is associated with increased transcription of multiple inflammatory genes [151, 152].

Another transcription factor that activates inflammatory genes is activator protein-1 (AP-1), a heterodimer of Fos and Jun proteins. As with NF-κB there are several redox-sensitive steps in the activation pathway [153]. Exogenous oxidants may also be important in worsening airway disease. Cigarette smoke, ozone and, to a lesser extent, nitrogen dioxide, impose an oxidative stress on the airways [154].

Oxidants also activate mitogen-activated protein (MAP) kinase pathways. H_2O_2 is a potent activator of extracellular regulated kinases and p38 MAP kinase pathways that regulate the expression of many inflammatory genes and survival in certain cells, and the spreading of macrophages [155]. Indeed many aspects of macrophage function are regulated by oxidants through the activation of multiple kinase pathways [156].

Evidence for increased oxidative stress

There is considerable evidence for increased oxidative stress in COPD [129, 130]. Cigarette smoke itself contains a high concentration of ROS [157]. Inflammatory cells, such as activated macrophages and neutrophils, also generate ROS, as discussed above. Epidemiological evidence indicates that reduced dietary intake of antioxidants may be a determinant of COPD and population surveys have linked a low dietary intake of the antioxidant ascorbic acid (vitamin C) with worse lung function [158, 159].

There are several markers of oxidative stress that may be detected in the breath and several studies have demonstrated increased production of oxidants in exhaled air or breath condensates [160–162]. There is an increased concentration of H_2O_2 in exhaled breath condensate of patients with COPD, particularly during exacerbations [163, 164].

There is also an increase in the concentration of 8-isoprostaglandin $F_{2\alpha}$ (8-isoprostane) in exhaled breath condensate, which is found even in patients who are exsmokers [165] and is increased further during acute exacerbations [166]. Isoprostane is also increased in the breath of normal smokers, but to a lesser extend than in COPD, suggesting that there is an exaggeration of oxidative stress in COPD. 8-Isoprostane is similarly increased in the urine of patients with COPD and further increased during exacerbations [167].

There is also evidence for increased systemic markers of oxidative stress in patients with COPD as measured by biochemical markers of lipid peroxidation [168]. A specific marker lipid peroxidation 4-hydoxy-2-nonenal which forms adducts with basic amino acid residues in proteins can be detected by immunocytochemistry and has been detected in lungs of patients with COPD [169]. This signature of oxidative stress is localised to airway and alveolar epithelial cells, endothelial cells and neutrophils.

Role of oxidative stress

The increased oxidative stress in the airways of COPD patient may play an important pathophysiological role in the disease by amplifying the inflammatory response in COPD. This may reflect the activation of NF-κB and AP-1, which then induce a neutrophilic inflammation *via* increased expression of IL-8 and other CXC chemokines, TNF-α and MMP-9. NF-κB is activated in airways and alveolar macrophages of patients with COPD and is further activated during exacerbations [73, 74]. It is likely that oxidative stress is an important activator of this transcription factor in COPD patients.

Oxidative stress may also impair the function of antiproteases such as α_1-antitrypsin and SLPI, and thereby accelerate the breakdown of elastin in lung parenchyma [170].

Corticosteroids are much less effective in COPD than in asthma and do not reduce the progression of the disease [171–174]. In contrast to patients with asthma, those with COPD do not show any significant anti-inflammatory response to corticosteroids [80, 81]. Alveolar macrophages from patients with COPD show a marked reduction in responsiveness to the anti-inflammatory effects of corticosteroids, compared to cells from normal smokers and nonsmokers [82]. Recent studies suggest that there may be a link between oxidative stress and the poor response to corticosteroids in COPD. Oxidative stress impairs binding of glucocorticoid receptors to DNA and the translocation of these receptors from the cytoplasm to the nucleus [175, 176]. Corticosteroids switch off inflammatory genes by recruiting HDAC2 to the active transcription site and by deacetylating the hyperacetylated histones of the actively transcribing inflammatory gene, they are able to switch off its transcription and thus suppress inflammation [84, 177]. In cigarette smokers and patients with COPD there is a marked reduction in activity of HDAC and reduced expression of HDAC2 in alveolar macrophages [83] and an even greater reduction in HDAC2 expression in peripheral lung tissue [178]. This reduction in HDAC activity is correlated with reduced suppression of inflammatory cytokines and a reduced response to corticosteroids. This may result directly or indirectly from oxidative stress and is mimicked by the effects of H_2O_2 in cell lines [178].

Oxidative stress may also induce apoptosis in endothelial and epithelial cells. Apoptosis of type 1 pneumocytes may be contributory to the development of emphysema and this

might be induced by cytotoxic T-lymphocytes or by inhibition of vascular-endothelial growth factor receptors [90, 123]. ROS may induce apoptosis by activating the NF-κB pathway, by direct DNA damage *via* activation of polyadenosine diphosphate ribose and *via* the generation of 4-hydroxy-nonenal. Apoptosis signal-regulating kinase-1 is held in an inactive conformation by thioredoxin and when oxidised by ROS this triggers apoptotic pathways [179].

Which proteases are important?

It has long been proposed that various proteases break down connective tissue components, particularly elastin, in lung parenchyma to produce emphysema and that there is an imbalance between proteases and endogenous antiproteases which should normally protect against protease-mediated effects. Elastin may be the most important target for these enzymes as there is a loss of elasticity in the lung parenchyma in patients with emphysema and elastin cannot be regenerated in an active form. Evidence for elastin degradation in COPD is provided by the increased excretion of desmosine, derived from elastin cross-links, in smokers with rapid decline in lung function compared to those with a normal decline [180]. Although early attention was focussed on neutrophil elastase (NE), many other proteases that have the capacity to degrade elastin have now been implicated [181].

Neutrophil elastase

There has been particular emphasis on the role of NE since patients with inherited α1-antitrypsin (α_1-AT) deficiency were shown to develop early onset emphysema. Furthermore the demonstration that α_1-AT may be inactivated by cigarette smoke exposure raised the possibility that neutrophil elastase may also be important in smokers with normal plasma α_1-AT concentrations. This was supported by animal models in which tracheal instillation of NE induces emphysema and infiltration of neutrophils [182] and immunocytochemical localisation of NE on elastin fibres in the lung parenchyma of patients with emphysema [183]. NE (E.C.3.4.21.37) is a serine protease which is inhibited by α_1-AT in the lung parenchyma. It is stored in azurophilic granules in neutrophils and in cells primed by cytokines it may be expressed on the cell surface [184].

NE has subsequently been shown to have several other actions relevant to its potential role in COPD. It is a potent mucus secretagogue of submucosal gland cells and goblet cells [40, 185]. NE induces the expression of MUC5AC in an epithelial cell line and this mechanism appears to be dependent on the generation of reactive oxygen species [186, 187]. NE also induces the expression of some cytokines, including IL-8 in airway epithelial cells [188]. NE cleaves the phosphatidylserine receptor on macrophages, thus impairing their ability to clear apoptotic cells [89].

On the other hand NE also inactivates CD14, a cell surface receptor for lipopolysaccharide, thus reducing the inflammatory response to endotoxin [189]. NE is likely to play a role in host defence and $NE^{(-/-)}$ mice have increased susceptibility to overwhelming Gram-negative bacterial infections, but do not appear to have any increase in spontaneous infections [190, 191].

The role of NE in COPD will only be established when the effect of NE inhibitors has been studied clinically [192]. In guinea pigs exposed to cigarette smoke a NE inhibitor markedly reduced emphysema and the neutrophil inflammatory response [193]. Although several NE inhibitors have been tested in humans there are few results reported. It is not certain whether the drugs failed or the clinical trials were not adequately designed. An NE inhibitor MR889 had no effect on urinary desmosine in unselected COPD patients, but a small reduction was seen in patients with a relatively short history [194]. The macrolide antibiotics erythromycin and flurithromycin have also been shown to inhibit NE activity [195] and this might account for their beneficial effect on mucus hypersecretion [196].

Other serine proteases

Neutrophils also store two other serine proteases cathepsin G and proteinase 3 in their specific granules. These other serine proteases have similar properties to NE and induce mucus secretion in a similar way [40, 42]. Proteinase 3 is potently expressed on the surface of neutrophils after activation with cytokines [197]. Proteinase 3 is potently inhibited by α_1-AT [198]. The neutrophil elastase inhibitors in development inhibit other serine proteases [192].

Cysteine proteases

Lysosomal cysteine proteases (cathepsins) may also be involved in COPD [199, 200]. Cathepsin S expression is induced by interferon-γ in several cell types, including smooth muscle cells. Overexpression of IFN-γ induces emphysema in mice and there is increased expression of cathepsins B, D, H, L and S [201]. Cathepsin inhibitors markedly reduce the emphysema-induced IL-13 transgenic mice, indicating the elastolytic potential of this cathepsin [202]. Several other cathepsins also have elastolytic activity, including cathepsins B, K and L, which are expressed in alveolar macrophages [69, 203] and cathepsin W in $CD8^+$ T-cells [204]. The role of cathepsins in COPD is uncertain. Increased concentrations of cathepsin L have been detected in BAL fluid of patients with emphysema [205] and alveolar macrophages from patients with COPD secrete more cysteine protease activity than macrophages from normal smokers or nonsmokers [70]. The endogenous inhibitors of cathepsins are cystatins and stefins, but little is known about their role in COPD. Cystatin C concentrations are increased in BAL fluid of patients with COPD [205].

Matrix metalloproteinases

There is increasing evidence for a role for MMPs in COPD [206]. In patients with emphysema there is an increase in BAL concentrations and macrophage expression of MMP-1 (collagenase) and MMP-9 (gelatinase B) [81, 207, 208]. There is an increase in activity of MMP-9 in the lung parenchyma of patients with emphysema [209]. MMP-1 expression is also increased in the lungs of patients with emphysema, with predominant localisation to type II pneumocytes [210]. Alveolar macrophages from normal smokers express more MMP-9 than those from normal subjects [72] and there is an ever greater increase in cells from patients with COPD [71], which have greatly enhanced elastolytic activity [70]. Indeed, using the MMP inhibitor marimastat it was shown that MMPs account for most of the elastolytic activity released from alveolar macrophages from COPD patients over prolonged periods [70].

The interest in MMPs has been heightened by the demonstration that emphysema induced by chronic cigarette exposure is prevented in $MMP\text{-}12^{-/-}$ (macrophage metalloelastase) mice [211]. In $MMP12^{-/-}$ mice emphysema induced by IL-13 and IFN-γ overexpression is reduced [201, 202] and there is a marked reduction in the recruitment of monocytes

into the lung. This may be because MMPs generate chemotactic peptides which promote macrophage recruitment to the parenchyma and airways. MMPs may activate the latent form of TGF-β to its active form. In addition, mice in which the integrin αvβ6 is deleted (Itgb6-null mice) fail to activate TGF-β and develop age-related emphysema which is prevented in $MMP12^{-/-}$ mice and by overexpression of TGF-β1 [212]. This suggests that TGF-β1 downregulates MMP-12 under normal conditions and absence of TGF-β results in excessive MMP-12 and emphysema. $MMP\text{-}9^{-/-}$ mice are not protected against emphysema induced by cigarette smoke, but are protected from small airway fibrosis [213]. TGF-β1 is activated by MMP-9 [214]; this may be mediated *via* MMP-9 induced proteolytic cleavage of latent TGF-binding protein-1, resulting in release of TGF-β1 [215]. This mechanism therefore could be a link between elastolysis induced by MMP-9 and simultaneous production of fibrosis by activation of TGF-β1 (fig. 3). Thus, MMP-12 is a prominent MMP in the mouse, and while present in humans does not appear to be as important as MMP-9.

Antiproteases

Normally proteases are counteracted by an excess of endogenous antiproteases. The major inhibitors of serine proteases are α_1-AT in lung parenchyma and airway epithelium-derived SLPI in the airways. Other serine protease inhibitors include elafin and α_1-antichymotrypsin. Serine protease inhibitors inactivate NE and other serine proteases such as proteinase-3 [216]. Multiple genetic variants of α_1-AT are now recognised that give rise to reduced circulating active α_1-AT concentrations [217, 218]. The best described deficiency that results in early onset emphysema is the ZZ type in which a single amino acid substitution (Gly342→Lys) results in structural alterations in α1-AT resulting in failure of its normal post-translational modification and secretion by hepatocytes leading to very low plasma concentrations. Whether heterozygotes and other genetic variants that reduce circulating α_1-AT concentrations to a lesser extent than the ZZ phenotype also predispose to emphysema is more debatable [219]. ZZ α_1-AT deficiency is a rare cause of emphysema accounting for <1% of patients, but it was proposed long ago that cigarette smoking may oxidise α_1-AT resulting in impaired antiprotease function and increased neutrophil elastase activity [220]. The mechanism appears to be due to oxidative stress and oxidation of methionine at positions 351 or 358 impairs anti-NE activity of α_1-AT [170].

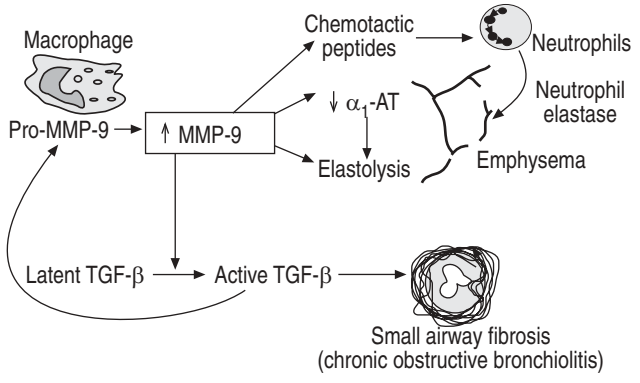

Fig. 3. – Possible interrelationship between small airway fibrosis and emphysema in chronic obstructive pulmonary disease. Transforming growth factor (TGF)-β activates matrix metalloproteinase-9 (MMP-9) and is in turn activated by MMP-9. α_1-AT: α_1-antitrypsin.

SLPI is the other major serine proteinase inhibitor in the airways [221]. Like α_1-AT, SLPI may be inactivated by oxidative stress, but also by cleavage through its active site by cathepsins L and S [222] and in patients with emphysema proteolytic fragments of SLPI are found in BAL fluid which contributes to the reduced anti-NE activity in these patients. This inactivation of SLPI not only impairs its anti-NE activity, but also its antimicrobial and anti-inflammatory roles. SLPI downregulates LPS-induced TNF-α and MMP secretion from monocytes [223, 224] and this may be mediated by an inhibitory effect on IκBα degradation, resulting in inhibition of NF-κB [225]. The role of elafin and α_1-antichymotryptase in COPD are less well defined [226, 227].

Four tissue inhibitors of MMPs (TIMP1-4) counteract MMPs [228]. TIMP-1 secretion from alveolar macrophages is increased in response to inflammatory stimuli, but the increase is blunted in cells derived from COPD patients, so favouring increased elastolysis [70, 71]. An increased frequency of loss of function mutations of TIMP-2 has been described in patients with COPD [229].

What are the amplifying mechanisms?

The inflammatory changes and protease imbalance in COPD are also seen in cigarette smokers without COPD but to a lesser extent [12, 47, 70, 81, 82], suggesting that the accelerated decline in lung function in COPD may be due to amplification of the normal pulmonary inflammatory response to irritants. This may be due to increased production of inflammatory mediators and enzymes, or because of defective endogenous anti-inflammatory or antiprotease mechanisms. These differences might be explained by polymorphisms in the genes encoding cytokines, proteases, anti-inflammatory proteins and antiproteases [230, 231].

Another hypothesis is that these differences are due to latent virus infection [232]. The latent adenovirus sequence E1A is more commonly detected in the lungs of patients with emphysema than in matched smoking control subjects and is correlated with an increased inflammatory response [47]. Adenovirus infection amplifies the inflammatory response to cigarette smoke in the airways of guinea pigs [68]. Transfection of E1A into a human epithelial cell line results in increased activation of the transcription factor NF-κB with consequent increased release of IL-8 in response to cell activation and increased production of TGF-β1, providing a molecular mechanism for the amplification in inflammatory response [233, 234].

Another molecular mechanism that may underlie the amplification of inflammation in COPD may involve impaired activity of HDAC in alveolar macrophages. In macrophages from cigarette smokers there is impaired activity of HDAC, which is involved in switching off the transcription of inflammatory genes by reversing the acetylation of core histones that is associated with their activation [83, 84]. In COPD patients there is even more marked reduction in HDAC activity in the peripheral lung than in smokers without airway obstruction [178]. This may lead to amplification of the expression of inflammatory genes, as is seen in alveolar macrophages from patients with COPD [71, 82]. There is also increased activation of NF-κB in these cells from patients with COPD [73, 74].

Although smoking is the major causal mechanism in COPD, quitting smoking does not appear to result in resolution of the inflammatory response in the airways, particularly in advanced disease [47, 235, 236]. This suggests that there are perpetuating mechanisms that maintain the chronic inflammatory process once it has become established. This may

account for presentation of COPD in patients who stopped smoking many years before their first symptoms develop. The mechanisms of disease persistence are currently unknown.

Why is there a poor response to corticosteroids?

Inhaled corticosteroids are now the mainstay of chronic asthma therapy and the recognition that chronic inflammation is also present in COPD provided a rationale for their use in COPD. Indeed, inhaled corticosteroids are now widely prescribed in the treatment of COPD and are used almost as frequently as in asthma. However, the inflammation in COPD is not suppressed even by high doses of inhaled or oral corticosteroids [80, 81, 237]. This may reflect the fact that neutrophilic inflammation in humans is not suppressible by corticosteroids as neutrophil survival is prolonged by steroids [238, 239]. Approximately 10% of patients with stable COPD show some symptomatic and objective improvement with oral corticosteroids and it is likely that these patients have concomitant asthma, as both diseases are very common. Indeed, airway hyperresponsiveness, a characteristic of asthma, may predict the rate of decline in COPD [240]. A response to corticosteroids is predicted by increased numbers of eosinophils in sputum and an increase in exhaled NO [105, 106], both characteristic features of asthma.

Four large studies have shown that high doses of inhaled corticosteroids fail to reduce the progression of COPD [171–174]. Inhaled corticosteroids may have a small effect in reducing exacerbations in patients with severe disease [241].

It seems likely that there is an active resistance to corticosteroids in COPD. High doses of corticosteroids fail to reduce cytokine and chemokines that should be suppressed by corticosteroid treatment [80, 81]. The molecular mechanisms of corticosteroid resistance are not yet known, but may be the same mechanisms that result in amplification of inflammatory responses. Thus a reduction in HDAC activity in macrophages [83] may prevent the anti-inflammatory action of corticosteroids, which is dependent on recruitment of HDACs to the inflammatory gene complex [84]. Similarly latent adenovirus appears to induce corticosteroid resistance in experimental animals [242].

By contrast, there is a beneficial effect of systemic corticosteroids in treating acute exacerbations of COPD, with improved clinical outcome and reduced length of hospital admission [243, 244]. The reasons for this discrepancy between steroid responses in acute *versus* chronic COPD may relate to differences in the inflammatory response (increased eosinophils) or airway oedema in exacerbations.

What are the mechanisms of acute exacerbations?

Although acute exacerbations of COPD, defined by increased symptoms and worsening lung function, are a common cause of hospital admission, their cellular and molecular mechanisms are far from clear [245]. Acute exacerbations may be prolonged and have a profound effect on the quality of life [246] and may accelerate the progression of COPD [247]. It was always assumed that the increased amount and purulence of sputum signified bacterial infection of the respiratory tract, but it is now evident that many exacerbations in COPD (as in asthma) are also due to upper respiratory tract viral infections (particularly rhinovirus) and to environmental factors, such as air pollution and temperature [248, 249] There is an increase in neutrophils and concentrations of IL-6, IL-8, TNF-α and LTB_4 in sputum during an exacerbation [250, 251] and patients who have frequent exacerbations have higher levels of IL-6 and lower concentrations of SLPI, even when COPD is stable [252, 253]. There is also an increase in the activation of NF-κB in alveolar macrophages during exacerbations of COPD, providing a link between infections, oxidative stress and the enhanced expression of inflammatory genes [74]. Purulent exacerbations are associated with bacterial infection and are characterised by a marked increase in LTB_4 concentrations in sputum [254]. Chronic bacterial colonisation of sputum is correlated with increased inflammatory indices in sputum [98] and predisposes to more frequent purulent exacerbations [255]. Bronchial biopsies show an increase in eosinophils during exacerbations in patients with mild chronic bronchitis [107] and this may reflect the marked upregulation of RANTES (regulated on activation, normal T-cell expressed and secreted) seen in airway epithelial cells during acute exacerbations [39]. However, there is no increase in sputum eosinophils during exacerbations in patients with severe COPD [252]. An increase in markers of oxidative stress and exhaled NO, presumably reflecting increased airway inflammation, are observed during exacerbations [163, 166, 256, 257]. This may reflect increased activation of NF-κB during exacerbations [74]. Thus exacerbations of COPD appear to be due to yet further amplification of the inflammatory process. This implies that treatments that suppress inflammation in COPD would also block exacerbations.

The reason why the lower respiratory tract of some patients with COPD is colonised with bacteria, such as nontypeable strains of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* is uncertain [258]. In a recent study 24% of 600 sputum specimens taken during an acute exacerbation contained a bacterial pathogen compared to 18% in patients without exacerbations [259]. Approximately one third of exacerbations were associated with "new" strains of bacteria with a differing protein composition, suggesting newly-acquired infection rather than a flare-up of existing organisms. However these "new" strains might arise from antigenic variants of persistent colonising strains, which may make them less susceptible to local immune mechanisms [260]. Persistence of certain bacteria in the lower respiratory tract of COPD patients may be due to localisation to protected sites. *H. influenzae* is localised to sites within the airway wall, such as between cells in the airway walls [261] and these organisms are not susceptible to antibiotics or antibody-mediated defence mechanisms when they are located between airway epithelial cells [262]. *H. influenzae* infection is associated with increased concentrations of inflammatory mediators, including IL-8 and TNF-α, in COPD patients [263]. The inflammatory process itself may also promote persistence of certain bacteria. For example, human neutrophil defensins may increase adhesion of *H. influenzae* to airway epithelial cells and may thus predispose to invasion [264].

What are the next questions?

The understanding of the cellular and molecular mechanisms involved in COPD is at an early stage compared with knowledge of asthma. Although both diseases involve inflammation in the respiratory tract, the pattern of inflammation, the results of the inflammatory process and the therapeutic response are markedly different. This review has focussed on some key questions that are now being addressed in COPD, although it is by no means comprehensive.

Although activation of several inflammatory cells has now been identified in COPD, their relative importance and sequential role in producing the typical pathology of COPD are still poorly understood. However, it is likely that cigarette

smoke and other irritants activate resident cells, including macrophages, epithelial cells and dendritic cells, which then signal the influx of other inflammatory cells, including neutrophils, monocytes and T-lymphocytes from the circulation. These cells all release multiple mediators of inflammation, although the pattern of mediators differs from those found in asthma. The pathological process is predominantly located in the lung periphery with involvement of small airways and lung parenchyma. However, the relationship between inflammation and fibrosis in small airways and destruction of lung parenchyma and mucus hypersecretion are uncertain and there are differences in the preponderance of these mechanisms between patients and at different stages of the disease. There is a need for better techniques for studying small airway function.

There appears to be an amplification of the inflammatory process between smokers who do not have airflow limitation and the minority of smokers with accelerated decline of lung function who develop COPD. The molecular basis for this amplification needs further investigation, but possible mechanisms relate to genetic differences in inflammatory, proteolytic or protective mechanisms or acquired latent viral infections. The mechanisms of amplification may also be linked to the relative steroid resistance found in COPD and a plausible link is deficiency in HDAC activation that both amplifies inflammatory gene expression and impairs the anti-inflammatory response to corticosteroids.

Much further research is now needed to answer some of these key questions. However, availability of tissues from patients with chronic obstructive pulmonary disease and patients who have a similar cigarette smoke exposure without chronic obstructive pulmonary disease and the development of novel molecular and cellular techniques make it likely that rapid progress will be made. This will make it possible to predict which smokers will develop chronic obstructive pulmonary disease and may identify new targets for the development of novel therapies that suppress this chronic inflammatory process. Many new drugs for chronic obstructive pulmonary disease are already in development [265] and several are about to enter clinical trials.

"***COPD: the important questions*" *a meeting held in Malta in November 2002***: Chairmen: P. Barnes (UK), R. Pauwels (Belgium) and S. Shapiro (USA); Participants: *Canada*: M. Cosio, J. Hogg; *Denmark*: J. Vestbo; *Ireland*: G. McElvaney; *Italy*: L. Fabbri , M. Luisetti; *Malta*: R. Ellul-Micalef; *The Netherlands*: L. van Alphen; *Switzerland*: L. Nicod; *UK*: P. Calverley, E. Chilvers, W. MacNee, N. Pride, W. Wedzicha; *USA*: H. Chapman, S. Kunkel, S. Rennard.

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