CHAPTER 8

Cells and mediators of chronic obstructive pulmonary disease

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Due to the enormous burden of disease and escalating healthcare costs, there is now renewed interest in the underlying cellular and molecular mechanisms of chronic obstructive pulmonary disease (COPD) [1, 2] and a search for new therapies [3]. The definition of COPD was adopted by the Global Initiative on Chronic Obstructive Lung Disease and for the first time this definition encompassed the idea that COPD is a chronic inflammatory disease [4]. Much of the recent research has focused on the nature of this inflammatory response.

COPD as an inflammatory disease

The progressive airflow limitation in COPD is due to two major pathological processes: 1) remodelling and narrowing of small airways; and 2) destruction of the lung parenchyma with consequent destruction of the alveolar attachments of these airways as a result of emphysema. This results in diminished lung recoil, higher resistance to flow and closure of small airways at higher lung volumes during expiration, thus, trapping air in the lung. This leads to the characteristic hyperinflation of the lungs, which gives rise to the sensation of dyspnoea and limits exercise capacity. The major symptom of COPD is shortness of breath on exertion. Both the small airway remodelling and narrowing and the emphysema are due to chronic inflammation in the lung periphery. Recent quantitative studies have shown that the inflammatory response in small airways and lung parenchyma increases as the disease progresses [5]. There is a specific pattern of inflammation in COPD airways and lung parenchyma, with increased numbers of macrophages, T-lymphocytes, with predominance of CD8+ (cytotoxic) T-cells, and, in more severe disease, B-lymphocytes with increased numbers of neutrophils in the lumen [2]. The inflammatory response in COPD involves both innate and adaptive immune responses. Multiple inflammatory mediators are increased in COPD and are derived from inflammatory cells and structural cells of the airways and lungs [6]. A similar pattern of inflammation is seen in smokers without airflow limitation. In COPD this inflammation is amplified and during acute exacerbations of the disease it is even further amplified, which is usually precipitated by bacterial and viral infections.

The molecular basis of this amplification of inflammation is not yet understood but may be partly genetically determined. Cigarette smoke and other irritants in the respiratory tract may activate surface macrophages and airway epithelial cells to release chemotactic factors, which then attract circulating leukocytes into the lungs. Amongst chemotactic factors chemokines predominate and, therefore, play a key role in

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orchestrating the chronic inflammation in COPD lungs and further amplification during acute exacerbations. These might be the initial inflammatory events occurring in all smokers. However, in smokers who develop COPD this inflammation progresses into a more complicated inflammatory pattern of adaptive immunity and involves T-cells, B-cells and probably dendritic cells, along with a complicated interacting array of cytokines and other mediators.

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 [7]. In COPD there is narrowing of bronchioles, with fibrosis and infiltration with macrophages and T-lymphocytes, along with destruction of lung parenchyma and an increased number of macrophages and T-lymphocytes, with a greater increase in CD8+ than CD4+ (helper) cells (fig. 1) [8]. Bronchial biopsies show similar changes with an infiltration of macrophages and CD8+ cells and an increased number of neutrophils in patients with severe COPD [9]. Bronchoalveolar lavage fluid (BALF) and induced sputum demonstrate a marked increase in macrophages and neutrophils [10, 11]. In contrast to asthma, eosinophils are not prominent except during exacerbations or when patients have concomitant asthma [7, 12].

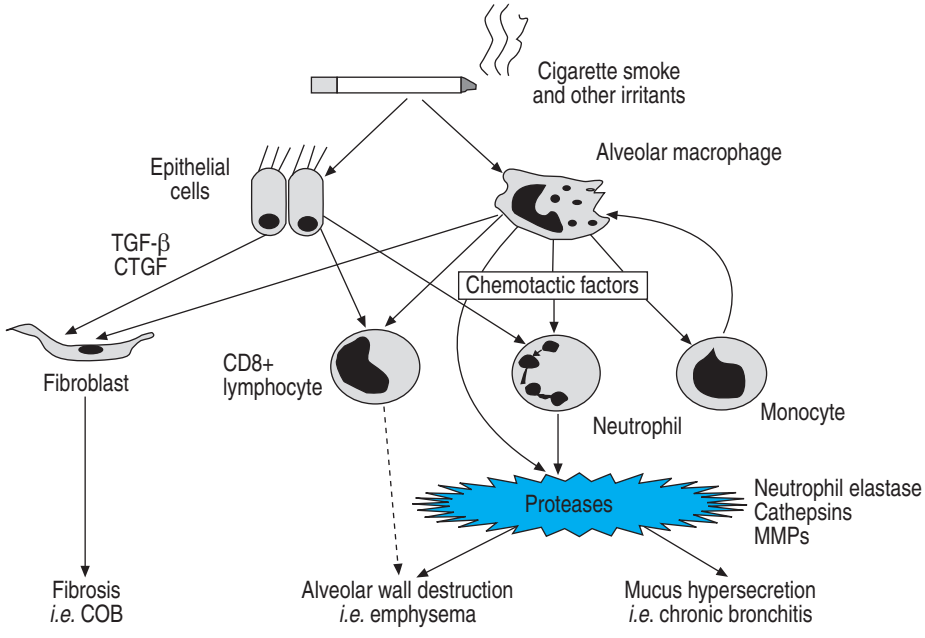

Fig. 1. – Inflammatory cells in chronic obstructive pulmonary disease. Cigarette smoke (and other irritants) activates macrophages in the respiratory tract releasing chemotactic factors. These then attract inflammatory cells from the circulation and fibrogenic factors, such as transforming growth factor (TGF-β) and connective tissue growth factor (CTGF) stimulating fibrosis in peripheral airways. Various cells release proteases in the airways, including matrix metalloproteinases (MMPs), which break down connective tissue in the lung parenchyma, resulting in emphysema and stimulate mucus hypersecretion. COB: chronic obstructive bronchitis.

Inflammatory cells

For many years it was believed that the inflammatory reaction in the lungs of smokers consisted of neutrophils and macrophages and that neutrophil's elastases and macrophage's proteinases were responsible for the lung destruction in COPD. This concept recently changed to include a more complicated inflammatory process after FINKELSTEIN *et al.* [13] described a prominent T-cell infiltration in the lungs of patients with COPD, which was strongly related to the extent of emphysema. Subsequent work by other authors confirmed these results and showed that the T-cells in the lungs of these patients were predominantly CD8+ T-cells, although CD4+ T-cells were also abundant. Further analysis of the cell profile in alveoli and small airways has shown an increase in all of the cell types implicated in COPD, including macrophages, T-lymphocytes, B-lymphocytes and neutrophils [14, 15].

Although abnormal numbers of inflammatory cells have been documented in COPD, the relationship between these cell types and the sequence of their appearance and persistence are not yet understood in detail [2]. Most studies have been cross-sectional based on a 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. Nonetheless there is a progressive increase in the number of inflammatory cells in small airways and lung parenchyma as COPD becomes more severe, even though patients with the most severe obstruction may have stopped smoking for many years [5]. This indicates the existence of some mechanisms that perpetuate the inflammatory reaction in COPD. This is in contrast to many other chronic inflammatory diseases, such as rheumatoid arthritis and interstitial lung diseases, where the inflammation tends to diminish in severe disease.

It is important to understand the inflammatory reaction to cigarette-smoke exposure, in order to realise that innate and adaptive immune responses are components of an integrated host-defence system, in which numerous cells and molecules function cooperatively. Two important links exist between innate and adaptive immunity. First, the innate immune response to microbes (or other offending molecules) stimulates adaptive immune responses and influences their nature. Secondly, adaptive immune responses use many of the effector mechanisms of innate immunity to eliminate microbes or other antigenic substances, and often function by enhancing the activities of the defence mechanisms of innate immunity. The innate immune system consists of epithelial barriers, circulating cells (neutrophils, macrophages, eosinophils, mast cells, natural killer (NK) cells, γ/δ-T-cells and dendritic cells) and proteins (complement), which recognise substances produced by infections or other foreign harmful substances and initiate responses that eliminate the offending agent [16].

Epithelial cells

Present evidence suggests that, by sending "danger" signals in response to cigarette smoke, the epithelium is responsible for the initiation and possible maintenance of the innate immune response seen in smokers, and 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 tumour necrosis factor (TNF)-α, interleukin (IL)-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF) and CXCL8 (IL-8) [17–19]. Epithelial cells in small airways may be an important source of transforming growth factor (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 receptor (VEGFR)2 in rats induces apoptosis of alveolar cells and an emphysema-like pathology [21]. The apoptosis of alveolar epithelial cells may be mediated *via* the sphingolipid ceramide [22]. Airway epithelial cells are also important in airways defence. Mucus produced from goblet cells traps bacteria and inhaled particulates [23]. Epithelial cells secrete defensins and other cationic peptides with antimicrobial effects and play a part in the innate defence system, but they are also involved in tissue-repair processes [24]. They also secrete antioxidants as well as antiproteases, such as secretory leukoprotease inhibitor (SLPI). Epithelial cells also transport immunoglobulin (Ig)A and are, therefore, also involved in adaptive immunity [25]. 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.

Another consequence of epithelium injury by cigarette smoke, and the resultant increase in epithelial permeability [26, 27], is the production and release of tachykinins (substance P and neurokinin A). The release of tachykinins from sensory nerves can be evoked by a variety of stimuli, including cigarette smoke, and modulate a number of important immunological functions, such as T-cell proliferation, lymphocyte traffic and cytokine production, including IL-1, IL-3, IL-6, IL-10, IL-12 and TNF-α [28, 29]. Thus, the bronchial epithelium, in addition to acting as a physicochemical barrier, plays a crucial role in initiating pulmonary host defence mechanisms, both in health and in disease, by synthesising and releasing a variety of mediators that can cause an innate immunity inflammatory cell differentiation, chemotaxis and cell activation.

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 proliferating cell nuclear antigen, is increased in some normal smokers, but is markedly increased in patients with chronic bronchitis and correlates with the degree of squamous metaplasia [30]. 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 (EGFR) 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 [31].

Neutrophils

Increased numbers of activated neutrophils are found in sputum and BALF of patients with COPD [11, 32], yet increase relatively little in the airways or lung parenchyma [13]. This may reflect their rapid transit through the airways and parenchyma. The role of neutrophils in COPD is not yet clear; however, there is a correlation between the number of circulating neutrophils and fall in forced expiratory volume in one second [33]. Neutrophil numbers in bronchial biopsies and induced sputum are correlated with COPD disease severity [9, 11] and with the rate of decline in lung function [34]. Smoking has a direct stimulatory effect on granulocyte production and release from the bone marrow and survival in the respiratory tract, possibly mediated by GM-CSF and granulocyte colony-stimulating factor released from lung macrophages [35]. Smoking may also increase neutrophil retention in the lung [36]. Neutrophil recruitment to the airways and parenchyma involves adhesion to endothelial cells and E-selectin, which is upregulated on endothelial cells in the airways of COPD patients [37]. Adherent neutrophils then migrate into the respiratory tract under the direction of neutrophil chemotactic factors. There are several chemotactic signals that have the potential for

neutrophil recruitment in COPD, including leukotriene (LT)B_4, CXCL8 and related CXC chemokines, including CXCL1 (growth-related oncogene-α (GRO-α)) and CXCL5 (ENA-78), which are increased in COPD airways [38, 39]. These mediators may be derived from alveolar macrophages, T-cells and epithelial cells, but it is possible that the neutrophil is a major source of CXCL8 [40]. Neutrophils from the circulation marginate in the pulmonary circulation and adhere to endothelial cells in the alveolar wall before passing into the alveolar space [41]. The 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 [42]. The cellular mechanisms underlying neutrophil adhesion and transmigration differ between systemic and pulmonary circulations, which 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; the upper lobe predominance in centrilobular emphysema, for example. Little is known about survival and apoptosis of neutrophils in COPD lungs. Theoretically, GM-CSF may prolong neutrophil survival but it has proved difficult to culture neutrophils from sputum samples.

The neutrophils recruited to the airways of COPD patients are activated as there are increased concentrations of granule proteins, such as myeloperoxidase (MPO) and human neutrophil lipocalin, in the sputum supernatant [43–45]. Neutrophils secrete serine proteases, including neutrophil elastase, cathepsin G and proteinase-3, as well as matrix metalloproteinase (MMP)-8 and MMP-9, which may contribute to alveolar destruction (fig. 2). 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 [46]. Neutrophils in the peripheral circulation

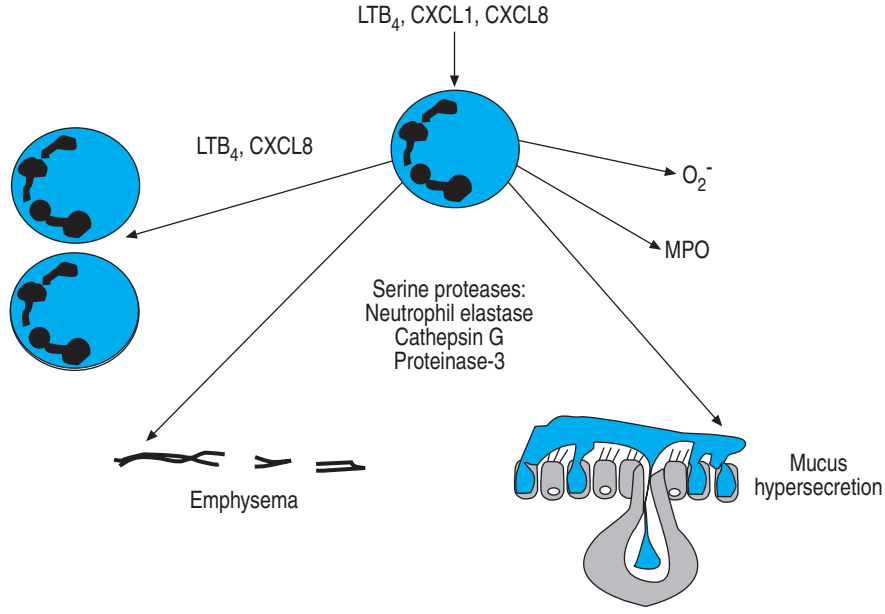

Fig. 2. – Neutrophils in chronic obstructive pulmonary disease. Neutrophils recruited to the lungs by chemotactic factors, such as leukotriene (LT)B_4 and the chemokines CXCL8 and CXCL1, are activated and release superoxide anions (O_2^-), myeloperoxidase (MPO), LTB_4, CXCL8 and serine proteases.

show evidence of priming in COPD [47], but this may result from rather than contribute to lung pathophysiology.

However, while neutrophils have the capacity to cause elastolysis, this is not a prominent feature of other pulmonary diseases in which 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 [13], and neutrophils are not a prominent feature of parenchymal inflammation in COPD. However, it is likely that airway neutrophilia is linked to mucus hypersecretion in chronic bronchitis. Serine proteases form 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 [48, 49].

There is a marked increase in neutrophil numbers in the airways in acute exacerbations of COPD accounting for the increased purulence of sputum. This may reflect increased production of neutrophil chemotactic factors, including LTB_4 and CXCL8 [50–52].

Macrophages

Macrophages appear to play a pivotal role in the pathophysiology of COPD and can account for most of the known disease features [53] (fig. 3). There is a marked increase (five to 10-fold) in the number of macrophages in airways, lung parenchyma, BALF and

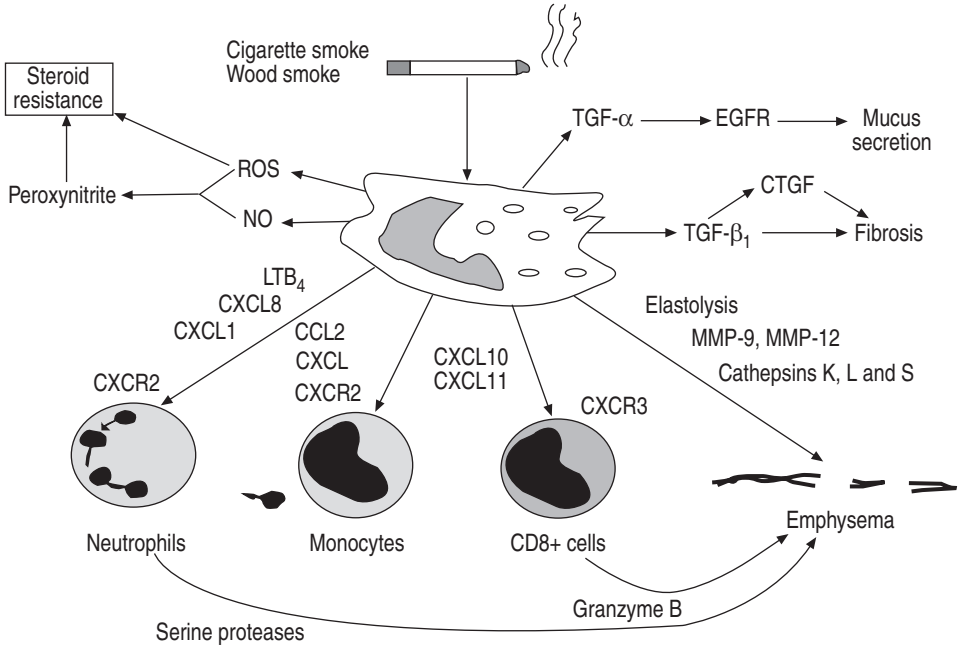

Fig. 3. – Macrophages in chronic obstructive pulmonary disease (COPD). Macrophages may play a pivotal role in COPD as they are activated by cigarette smoke extract and secrete many inflammatory proteins, which may orchestrate the inflammatory process in COPD. Neutrophils may be attracted by CXCL8, CXCL1 and leukotriene (LT)B_4, monocytes by CCL2, and CD8+ lymphocytes by CXCL10 and CXCL11. Release of elastolytic enzymes, including matrix metalloproteinases (MMPs) and cathepsins, causes elastolysis and the release of transforming growth factor (TGF)-β_1 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. EGFR: epidermal growth factor receptor.

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 [14]. Furthermore, macrophages are localised to sites of alveolar wall destruction in patients with emphysema [13, 54]. There is a correlation between macrophage numbers in the parenchyma and airways, and between the severity of emphysema [13] and COPD [9].

Macrophages may be activated by cigarette-smoke extract to release inflammatory mediators, including TNF-α, CXCL8 and other CXC chemokines, CCL2 (monocyte chemotactic protein-1), LTB_4 and reactive oxygen species (ROS), 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 [55, 56]. 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 [56–58]. 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 [56]. 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 (NF)-κB, which is activated in alveolar macrophages of COPD patients, particularly during exacerbations [59, 60].

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 CCL2 is increased in sputum and bronchoalveolar lavage (BAL) of patients with COPD [38, 61], with increased expression in macrophages [62]. CXC chemokines are also chemoattractant to monocytes acting *via* CXCR2 and the concentration of CXCL1 is markedly increased in sputum and BAL of patients with COPD [38]. 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 [63]. Interestingly, while all monocytes express CCR2, the receptor for CCL2, 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 release the chemokines CXCL9 (monokine induced by interferon-γ), CXCL10 (interferon-γ inducible protein of 10 kDa) and CXCL11 (interferon-inducible T-cell-α chemoattractant), which are chemotactic for CD8+ Tc1 and CD4+ T-helper (Th)-1 cells *via* interaction with the chemokine receptor CXCR3 expressed on these cells [64, 65].

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 it has been demonstrated that there is some increase in cell proliferation measured by proliferative cell nuclear antigen [66]. 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 [66]. This suggests that macrophages may have a prolonged survival in smokers and patients with COPD. It is very likely that the increased activity and survival of macrophages is mediated by T-cells. One of the main functions of the effector Th1 and T cytotoxic (Tc)1 T-cells is the activation of alveolar macrophages. This is mediated by interferon (IFN)-γ and the expression of CD40 ligand. Once activated, macrophages will increase production of reactive oxygen intermediates, nitric oxide (NO) and lysosomal enzymes and will increase secretion of many cytokines, including TNF-α, IL-1β, IL-6,

CXCL8 and IL-18 among others. Activated macrophages are aimed at the more efficient killing of organisms and promote further inflammation, mainly by TNF-α, IL-1β and short-lived lipid mediators. In addition to their effector functions, activated macrophages become more efficient antigen-presenting cells by increasing the major histocompatability (MHC) class II expression and stimulating of T-cell proliferation and differentiation, such as IL-12 and IL-18 [67].

Corticosteroids are ineffective in suppressing inflammation, including cytokines, chemokines and proteases, in patients with COPD [68, 69]. *In vitro*, the release of CXCL8, 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 [70]. 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) [71–73], which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes [74, 75]. The reduction in HDAC activity in macrophages is correlated with increased secretion of cytokines, such as TNF-α and CXCL8, and reduced response to corticosteroids. The reduction of HDAC activity on COPD patients may be mediated through oxidative stress and peroxynitrite formation [76].

Eosinophils

While eosinophils are the predominant leukocyte in asthma, their role in COPD is much less certain. Increased numbers of eosinophils have been described in the airways and BAL of patients with stable COPD, whereas others have not found increased numbers in airway biopsies, BAL or induced sputum [77]. The presence of eosinophils in patients with COPD predicts a response to corticosteroids and may indicate coexisting asthma [78, 79]. Increased numbers of eosinophils have been reported in bronchial biopsies and BALF during acute exacerbations of chronic bronchitis [80–82]. 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 [43]. This may be due to the high levels of neutrophil elastase that have been shown to cause degranulation of eosinophils [83].

NK cells

NK (CD56+) cells are the first-line defence against viral infections. Circulating NK cells are reduced in patients with COPD and have reduced phagocytic activity [84]. Similar findings are noted in normal smokers [85], although no difference in NK cells was found in lung parenchyma of COPD patients. There is an increase in γ/δ T-cells in alveoli of smokers, whether they have airway obstruction or not [86].

Dendritic cells

Dendritic cells (DCs) play a central role in the initiation of the innate and adaptive immune response and it is believed that DCs provide a link between them [87]. The airways and lungs contain a rich network of DCs that are localised near the surface, so that they are ideally located to signal the entry of inhaled foreign substances. Recruitment of a wave of DCs into the respiratory tract mucosa is a universal feature of the acute cellular response to local challenge with bacterial, viral and soluble protein

antigens [88]. This suggests that rapid amplification of specific antigen surveillance at peripheral challenge sites is an integral feature of the innate immune response and serves as an "early warning system" to alert the adaptive immune system to incoming pathogens or body injury. DCs can activate a variety of other inflammatory and immune cells, including macrophages, neutrophils and T- and B-lymphocytes [89]. Therefore, it is likely that the DCs may play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents.

There is an increase in the number of DCs in rat lungs exposed to cigarette smoke [90]. Cigarette smoking is associated with an expansion in the DC population in the lower respiratory tract [91] and with a marked increase in the number of mature cells in the airways and alveolar walls of smokers [92]. This is an indication that the lung response to cigarette-smoke exposure follows the established immune response design, including innate immunity and readiness for an adaptive immune response, if necessary. DCs respond to two types of signals: 1) direct recognition of pathogens; and 2) danger signals *via* inflammatory cytokines, internal cellular signals and ongoing specific immune responses. The stimulation of a variety of surface receptors on DCs trigger cell maturation and antigen presentation by pathogenic compounds, inflammatory mediators, such as TNF-α, IL-1β, prostaglandin (PG)E_2, GM-CSF and Ig, heat shock proteins released by necrotic and injured cells, T-cell-derived signals (mainly CD4OL), and both necrotic and apoptic cell death [67]. Interestingly, an α-glycoprotein isolated from tobacco has powerful immunostimulatory actions [93].

The mechanism by which tobacco smoke activates the immune system is not yet understood, but the innate immune reaction in smokers has been shown to be accompanied by many of the inflammatory mediators listed previously and, along with products derived from the cigarette-smoke injured lung, could easily provide the necessary co-stimulation for DC maturation and eventual activation of the adaptive immune system (T- and B-cells). Pulmonary histiocytosis is a disease caused by DC granulomata in the lung and is characterised by destruction of the lung parenchyma that resembles emphysema [94]. The adult form of the disease occurs almost exclusively in smokers. The role of DCs in recruiting other effector cells in COPD deserves further study.

T-lymphocytes

Based on the present knowledge of the immune system (inflammation) and the interaction of the innate and adaptive immune systems towards fighting an attack on the host, the presence of T-cells in COPD is an expected finding. Furthermore, it would have been surprising if T-cells had not been part of the inflammatory component of the disease.

There is an increase in the total numbers of T-lymphocytes in lung parenchyma, peripheral and central airways of patients with COPD, with the greatest increase in CD8+ rather than CD4+ cells [5, 13, 14, 95–97]. There is a correlation between the number of T-cells and the amount of alveolar destruction, and the severity of airflow obstruction. Furthermore, the only significant difference in the inflammatory cell infiltrate in asymptomatic smokers and smokers with COPD is an increase in T-cells, mainly CD8+, in patients with COPD [86, 95]. There is also an increase in the absolute number of CD4+ T cells, albeit in smaller numbers, in the airways of smokers with COPD. These cells express activated signal transducer and activator of transcription (STAT)-4, a transcription factor that is essential for activation and commitment of the Th1 lineage and IFN-γ [98].

The ratio of CD4+ to CD8+ cells is reversed in COPD. This is mainly found in smokers with COPD rather than smokers without evidence of airflow limitation. The majority of T-cells in the lung in COPD are of the Tc1 and Th1 subtypes [64, 65]. CD8+ and CD4+ T-cells show increased expression of activation markers compared with

T-cells in the circulation, although there is no clear difference between patients with COPD and normal controls [99]. There is a marked increase in T-cells in the walls of small airways in patients with severe COPD and the T-cells are formed into lymphoid follicles, surrounding B-lymphocytes [5].

The mechanisms by which CD8+ and, to a lesser extent, CD4+ cells accumulate in the airways and parenchyma of patients with COPD is not yet understood [100]. However, homing of T-cells to the lung must depend upon some initial activation (only activated T-cells can home to the organ source of antigenic products), then adhesion and selective chemotaxis. Imprinting, or selection, for tissue differential homing properties is determined by the local lymphoid organ microenvironment and begins almost immediately during the DC-mediated naïve-to-memory/effector T-cell transition [67]. Homing receptor regulation during memory effector T-cell differentiation is analogous to (and temporally concomitant with) effector T-cell cytokine production (*i.e.* IFN-γ, IL-2 in the Th1 subset) involving immunoregulatory cytokines, as well as the nature of antigenic and co-stimulatory signals. As lymphocytes must be positioned correctly to interact with other cells, the pattern of chemokine receptors, and the type and distribution of chemokines in tissues, will critically influence immune response [67].

CD4+ and CD8+ T-cells in the lungs of COPD patients show increased expression of CXCR3, a receptor activated by the chemokines CXCL9, CXCL10 and CXCL11. There is increased expression of CXCL10 by bronchiolar epithelial cells and this could contribute to the accumulation of CD4+ and CD8+ T-cells, which preferentially express CXCR3 (fig. 4) [64]. The T-cells in COPD do not express any of the chemokine receptors

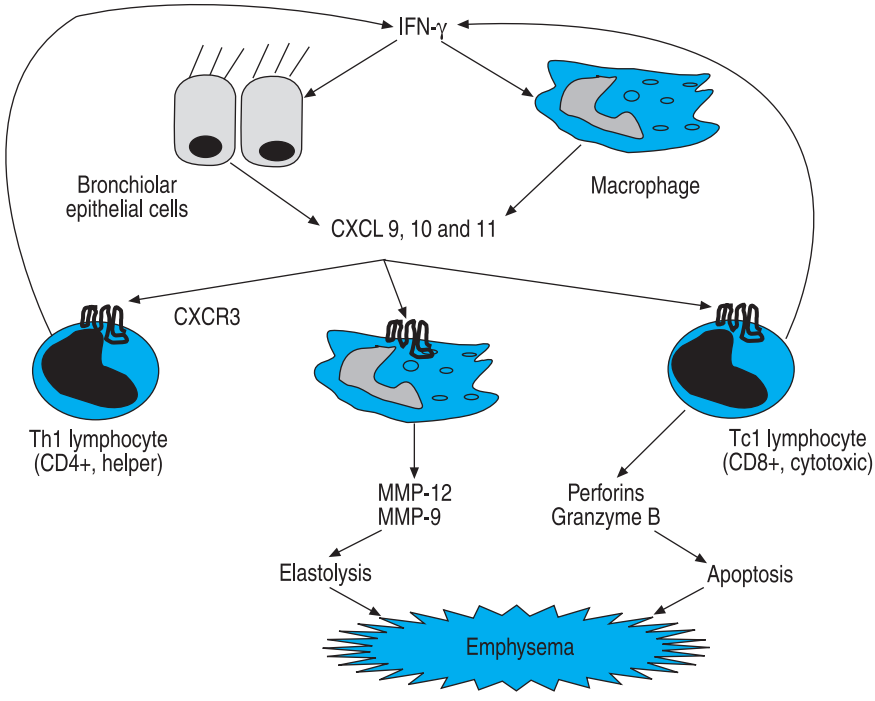

Fig. 4. – T-lymphocytes in chronic obstructive pulmonary disease. Chemotaxis of CD8+ T-lymphocytes (Tc1) and CD4+ cells (T-helper; Th1) *via* activation of CXCR3 by the CXC chemokines CXCL9, CXCL10 and CXCL11. CD8+ cells may release perforins and granzyme B, which may induce apoptosis in alveolar cells and release interferon (IFN)-γ which in turn activates the release of these chemokines. CXC3 chemokines also activate macrophages to release matrix metalloproteinases (MMP).

described in asthma (CCR4 and CCR8), indicating that the infiltrating T-cells in COPD are activated, Th1 committed, utilise Th1-type chemokines and receptors to home to the lung [101], and are likely to use Th1 cytokines and functions (cytolysis) as effector tools to damage the lung tissue. These results are a strong indication that the T-cells in COPD that express phosphorylated STAT-4 and IFN-γ are effector cells, activated by antigenic peptides from the lung in the local lymphoid tissue and homing back to the lung, the source of the antigens guided by Th1-selective chemokines. The findings are another indication of an adaptive immune response taking place in the lung, probably as a response to cigarette-smoke exposure and mediated tissue injury. The adaptive immune response could in turn increase and perpetuate tissue injury.

There is also an increase in the number of CD8+ cells in the circulation in COPD patients who do not smoke [102, 103] and an increase in Th1 type (IFN-γ-producing) CD4+ cells in smokers with COPD [65, 104]. This indicates that there may be chronic immune stimulation *via* antigens cross-presented by DCs that may migrate from the airways to regional lymph nodes, *via* the human leukocyte antigen class I and also class II pathways, which would stimulate the activation and proliferation of CD8+ and CD4+ T-cells, respectively. 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 [105]. It is possible that cigarette-induced lung injury may uncover previously sequestered auto-antigens or cigarette smoke itself may damage lung interstitial and structural cells, making them antigenic [106]. The role of increased numbers of CD4+ cells in COPD, particularly in severe disease, is also unknown [14]; however, it is now clear that T-cell help is required for the priming of cytotoxic T-cell responses, for maintaining CD8+ T-cell memory and for ensuring CD8+ T-cell survival [67]. Thus, the presence of CD4+ T-cells seems to be essential for the maintenance of a CD8+ inflammation and their effector functions. It is also possible that CD4+ T-cells have immunological memory and play a role in perpetuating the inflammatory process in the absence of cigarette smoking. In a mouse model of cigarette-induced emphysema there is a predominance of T-cells that are directly related to the severity of emphysema [107].

The role of T-cells in the pathophysiology of COPD is not yet certain, although they have the potential to produce extensive damage in the lung. CD8+ cells have the capacity to cause cytolysis and apoptosis of alveolar epithelial cells through the release of perforins, granzyme-B and TNF-α [108, 109]. There is an association between CD8+ cells and apoptosis of alveolar cells in emphysema [86]. Apoptotic cells are powerful sources of antigenic material that could reach the DC and perpetrate the T-cell response. CD8+ T-cells also produce a number of cytokines of the Tc1 phenotype, including TNF-α, lymphotoxin (TNF-β) and IFN-γ, and there is evidence that CD8+ in the lungs of COPD patients expresses IFN-γ [67]. All these cytokines would enhance the inflammatory reaction in the lung besides the direct killing by CD8+ cells.

The effector functions of the CD4+ T-cell are mainly mediated by Th1 cytokines. Essentially once T-cells (CD4+ and CD8+) are activated and home to the lung they stimulate much greater leukocyte migration, the so-called "immune inflammation" by the production of TNF-α and chemokines, ligands for leukocyte adhesion molecules, vasodilatory substances (VEGF, prostacyclin) and coagulation factors that would facilitate the entry of leukocytes to the site of injury. One of the main functions of the effector Th1 (and Tc1) T-cells is the activation of alveolar macrophages mediated by IFN-γ and the expression of CD40 ligand. Once activated, macrophages will increase production of reactive oxygen intermediates, NO and lysosomal enzymes and will increase secretion of many cytokines, including TNF-α, IL-1β and IL-18, among others [67].

It is now apparent that the inflammatory process leading to disease in COPD cannot be focused on one single cell. Each cell has its role or roles in the complex inflammatory

and immune process, but there is necessary and important cooperation among all the cells involved, which can be orchestrated best by the T-cells, as previously discussed. The rest of the inflammatory cells, besides being effector arms under the direction of the T-cells, enhance and maintain the T-cell function by providing the necessary inflammatory milieu for the maintenance of T-cell activation and co-stimulation.

There is now overwhelming evidence showing the presence of activated T-cells in the lungs in COPD patients. According to the present concepts of T-cell physiology [67], if the T-cells, alone or together with other inflammatory cells, were responsible for the lung injury and progression of COPD, it would be as a response to an antigenic stimulus originating in the lung. Hence, COPD would have to be considered an autoimmune disease triggered by smoking, as previously suggested [106, 110–112]. In favour of this hypothesis is the recently published evidence that the lungs of patients with severe emphysema contain highly activated oligoclonal T-cells [113]. These findings strengthen the hypothesis that cellular-mediated immunity plays a critical role in the pathogenesis of severe emphysema [114]. Furthermore, emphysema has been produced in animals by adoptive transfer into naïve immunocompetent rats of T-cells from rats which developed emphysema after *i.p.* injection of foreign endothelial cells. Adoptive transfer of disease by T-cells is proof of an immune mechanism in COPD [114].

Mediators of inflammation

Many inflammatory mediators have now been implicated in COPD, including lipids, free radicals, cytokines, chemokines and growth factors [6]. These mediators are derived from inflammatory and structural cells in the lung and interact with each other in a complex manner.

Lipid mediators

The profile of lipid mediators in exhaled breath condensates of patients with COPD shows an increase in PGs and leukotrienes [115]. There is a significant increase in PGE_2 and $F_{2\alpha}$ and an increase in LTB_4 but not cysteinyl leukotrienes. This is a different pattern to that seen in asthma, in which increases in thromboxane and cysteinyl leukotrienes have been shown [116]. The increased production of prostanoids in COPD is likely to be secondary to the induction of cyclo-oxygenase-2 (COX2) by inflammatory cytokines. Increased expression of COX2 is found in alveolar macrophages of COPD patients [117]. LTB_4 concentrations are also increased in induced sputum [118] and are further increased in sputum and exhaled breath condensate during acute exacerbations [50, 51]. LTB_4 is a potent chemoattractant of neutrophils, acting through high-affinity BLT_1-receptors. A BLT_1-receptor antagonist reduces the neutrophil chemotactic activity of sputum by ~25% [119]. Recently, BLT_1-receptors have been identified on T-lymphocytes and there is evidence that LTB_4 is involved in recruitment of T-cells [120].

Oxidative stress

Oxidative stress occurs when ROS are produced in excess of the antioxidant defence mechanisms resulting in harmful effects, including damage to lipids, proteins and DNA. There is increasing evidence that oxidative stress is an important feature in COPD [121, 122].

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including neutrophils, eosinophils, macrophages and epithelial

cells [121]. Superoxide anions ($O_2^{\cdot-}$) are generated by reduced nicotinamide adenine dinucleotide phosphate 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 (OH). $O_2^{\cdot-}$ may also combine with NO to form peroxynitrite, which also generates OH [123]. 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 [124], including bronchoconstriction and plasma exudation (fig. 5) [125].

Granulocyte peroxidases, such as MPO in neutrophils, play an important role in oxidative stress. In neutrophils, H_2O_2 generated from superoxide anions ($O_2^{\cdot-}$) is metabolised by MPO in the presence of chloride ions to hypochlorous acid, which is a strong oxidant. MPO is also able to nitrate tyrosine residues, as can peroxynitrite [126, 127].

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract [128]. The major intracellular antioxidants in the airways are catalase, superoxide dismustase (SOD) and glutathione, formed by the enzyme γ-glutamyl cysteine synthetase and glutathione synthetase. Oxidative stress activates the inducible enzyme haem oxygenase (HO)-1, converting haem and hemin to biliverdin with the formation of carbon monoxide (CO) [129]. Biliverdin is converted *via* bilirubin reductase to bilirubin, which is a potential antioxidant. HO-1 is widely expressed in human airways [130] and CO production is increased in COPD [131]. In the lung,

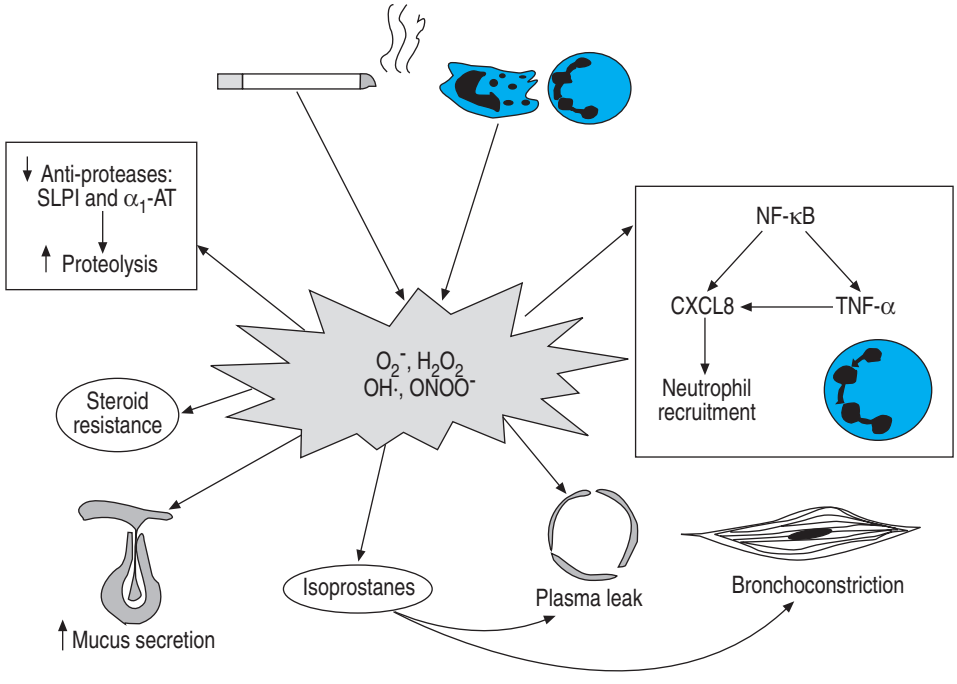

Fig. 5. – Oxidative stress in chronic obstructive pulmonary disease (COPD). Oxidative stress plays a key role in the pathophysiology of COPD and amplifies the inflammatory and destructive process. Reactive oxygen species from cigarette smoke or from inflammatory cells (particularly macrophages and neutrophils) result in several damaging effects in COPD, including: decreased anti-protease defences, such as α_1-antitrypsin (α_1-AT) and secretory leukoprotease inhibitor (SLPI); activation of nuclear factor (NF)-κB resulting in increased secretion of the cytokines CXCL8 and tumour necrosis factor (TNF)-α; increased production of isoprostanes; and direct effects on airway function. In addition recent evidence suggests that oxidative stress induces steroid resistance.

intracellular antioxidants are expressed at relatively low levels and are not induced by oxidative stress, whereas the major antioxidants are extracellular [132]. 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 [128] and concentrations are further 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 [133]. eGPx inactivates H_2O_2 and O_2^- but may also reactivate nitrogen species [132]. Extracellular antioxidants also include the dietary antioxidants vitamin C (ascorbic acid) and vitamin E (α-tocopherol), uric acid, lactoferrin and extracellular SOD (SOD3). SOD3 is highly expressed in human lung but its role in COPD is not yet clear [134].

ROS have several effects on the airways and parenchyma, 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 and alveoli 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. 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 [135]. 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 [136, 137]. Another transcription factor that activates inflammatory genes is activator protein (AP)-1 and there are several redox-sensitive steps in the activation pathway [138]. 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. Oxidants also activate mitogen-activated protein kinase (MAPK) pathways. H_2O_2 is a potent activator of extracellular regulated kinases and p38 MAPK pathways, which regulate the expression of many inflammatory genes, survival in certain cells, and spreading of macrophages [139]. Indeed, many aspects of macrophage function are regulated by oxidants through the activation of multiple kinase pathways [140].

There is considerable evidence for increased oxidative stress in COPD [121, 122]. Cigarette smoke itself contains a high concentration of ROS. Inflammatory cells, such as activated macrophages and neutrophils, also generate ROS, as previously discussed. There are several markers of oxidative stress that may be detected in the breath and several studies have demonstrated increased production of oxidants, such as H_2O_2, 8-isoprostane and ethane, in exhaled air or breath condensates [141–143], particularly during exacerbations [51, 141].

There is also evidence for increased systemic markers of oxidative stress in patients with COPD, as measured by biochemical markers of lipid peroxidation. 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 [144]. This signature of oxidative stress is localised to airway and alveolar epithelial cells, endothelial cells and neutrophils.

The increased oxidative stress in the lung epithelium of the 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 CXCL8 (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 [59, 60].

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 accelerates the breakdown of elastin in lung parenchyma [145].

Corticosteroids are much less effective in COPD than in asthma and do not reduce the progression of the disease. In contrast to patients with asthma, those with COPD do not show any significant anti-inflammatory response to corticosteroids [68, 69, 146, 147]. Alveolar macrophages from patients with COPD show a marked reduction in responsiveness to the anti-inflammatory effects of corticosteroids, compared with cells from normal smokers and nonsmokers [70]. Recent studies suggest that there may be a link between oxidative stress and the poor response to corticosteroids in COPD. 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 [75]. In cigarette smokers and patients with COPD there is a marked reduction in activity of HDAC and reduced expression of HDAC2 in alveolar macrophages and peripheral lung tissue [72]. This reduction in HDAC activity is correlated with reduced expression 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 [76].

Nitrative stress

The increase in exhaled NO is less marked in COPD than in asthma, partly because cigarette smoking reduces exhaled NO [131, 148] and it is further increased during exacerbations [148, 149]. Recently, exhaled NO has been partitioned into central and peripheral portions showing reduced NO in the bronchial fraction but increased NO in the peripheral fraction, which includes lung parenchyma and small airways [150]. The increased peripheral NO in COPD patients may reflect increased expression of inducible NO synthase in epithelial cells and macrophages of patients with COPD [151, 152]. NO and superoxide anions combine to from peroxynitrite. This is unstable and degraded to nitrate, which is increased in exhaled breath condensate of COPD patients [153]. Peroxynitrite also nitrates certain tyrosine residues in proteins and there is increased expression of 3-nitrotyrosine in peripheral lung and macrophages of COPD patients [151, 152]. There is tyrosine nitration of HDAC2, which may lead to impaired activity and degradation of this enzyme, resulting in steroid resistance [76].

There is extensive literature investigating the possible role of environmental agents, in general, and ROS, in particular, in the production of autoimmune reactions [154]. Among the important protein modifiers present in smokers are free radicals/oxidative stress. Both NO by itself or combined with super-oxide to form the potent oxidising agent peroxynitrite and other ROS, can be strong protein modifiers and thus antigen producers. NO and ROS may affect different cellular functions and result in cell death, together with mitochondrial damage, DNA strand breaks and structural/functional modification of proteins [154]. Oxidative modification of proteins has been implicated in the immune mechanism of various diseases, such as rheumatoid arthritis, multiple sclerosis, autoimmune anti-phospholipid antibody syndrome, diabetes mellitus and, lately, atherosclerosis, in which epitopes generated in the process of atherogenesis, such as those produced by the oxidation of low-density lipoproteins, have been implicated as targets of autoimmunity [155, 156]. This is so far the clearest example of how modified self-proteins can become antigenic and produce disease. A common conclusion, easily applicable to cigarette smoking, is that ROS have a great potential for altering

self-proteins, which could then be recognised as antigens by the adaptive immune system. Thus, a modified self-determinant could have the ability to elicit an autoimmune T-cell response, while the self-determinant could not.

Inflammatory cytokines

Cytokines are the mediators of chronic inflammation and several have been implicated in COPD [6, 157, 158]. There is an increase in concentration of TNF-α in induced sputum in stable COPD with a further increase during exacerbations [11, 52]. TNF-α production from peripheral blood monocytes is also increased in COPD patients and has been implicated in the cachexia and skeletal muscle apoptosis found in some patients with severe disease [159]. TNF-α is a potent activator of NF-κB and this may amplify the inflammatory response. Currently, anti-TNF therapies are being assessed in COPD patients. IL-1β is another pro-inflammatory cytokine that may amplify the inflammation in COPD through the activation of similar, but not identical, signal transduction pathways and transcription factors, as TNF-α and IL-6 concentrations are also elevated in COPD sputum and, probably more importantly, in the systemic circulation [160] Although the role of IL-6 in COPD is far from certain it deserves further attention as it could possibly account for many of the features of the disease. IL-6 is produced by immune cells, including monocytes and lymphocytes usually in response to TNF-α, IL-1β and oxidative stress and has potent pro-inflammatory functions, which promote the persistence of the inflammatory process. It also promotes autoimmunity by, among other mechanisms, suppressing the production of CD25+, CD4+ regulatory cells. An interesting feature of this cytokine is that whereas most other cytokines function *via* paracrine/autocrine mechanisms, the major effects of IL-6 are a consequence of its presence in the circulation, as has been shown in COPD, and can take place at sites distant from its origin. One of the most important effects of the high blood levels of IL-6 is weight loss mainly secondary to muscle wasting, a prominent feature in severe COPD [161] There is an increase in Tc1 and Th1 cells in COPD airways and both of these subtypes of T-cell produce IFN-γ, which in turn activates macrophages and the expression of particular chemokines that attract more T-cells [65, 100].

Chemokines

Chemokines are small chemotactic cytokines that play a key role in the recruitment and activation of inflammatory cells through specific chemokine receptors. Several chemokines have now been implicated in COPD and are of particular interest, since chemokine receptors are G-protein coupled receptors, for which small molecule antagonists have now been developed [162].

CXCL8 concentrations are increased in induced sputum of COPD patients and increase further during exacerbations [11, 52, 118]. Indeed, there is a correlation between sputum CXCL8 concentrations and disease severity [44]. CXCL8 is secreted from macrophages, T-cells, epithelial cells and neutrophils. CXCL8 activates neutrophils *via* low affinity-specific receptors CXCR1, and is chemotactic for neutrophils *via* high affinity-receptors CXCR2, which are also activated by related CXC chemokines, such as CXCL1. CXCL1 concentrations are markedly elevated in sputum and BALF of COPD patients and this chemokine may be more important as a chemoattractant than CXCL8, acting *via* CXCR2, which is expressed on neutrophils and monocytes [38]. CXCL1 induces significantly more chemotaxis of monocytes of COPD patients compared with those of normal smokers and this may reflect increased turnover and recovery of CXCR2 in monocytes of COPD patients [63]. CXCL5 shows a marked increase in expression in

airway epithelial cells during exacerbations of COPD and this is accompanied by a marked upregulation of epithelial CXCR2 (fig. 6) [163].

CCL2 is increased in concentration of COPD sputum and BALF [38] and plays a role in monocyte chemotaxis *via* activation of CCR2. CCL2 appears to cooperate with CXCL1 in recruiting monocytes to the lungs. CCL1 is also increased in concentration in COPD patients and mediates chemotaxis of monocytes and neutrophils *via* CCL1. The chemokine CCL5 (RANTES; regulated on activation, normal T-cell expressed and secreted) is also expressed in airways of COPD patients during exacerbations and activates CCR5 on T-cells and CCR3 on eosinophils, which may account for the increased eosinophils and T-cells in the wall of large airways that have been reported during exacerbations of chronic bronchitis [82]. RANTES-mediated chemokine amplification in DCs may prolong inflammatory responses, shape the microenvironment and potentially enhance acquired and innate immune responses [164]. As discussed previously, CXCR3 are upregulated on Tc1 and Th1 cells of COPD patients with increased expression of their ligands CXCL9, CXCL10 and CXCL11. These chemokines are regulated by IFN-γ, which is released from these T-cell subtypes, forming a self-perpetuating network.

Growth factors

Several growth factors have been implicated in COPD and mediate the structural changes that are found in the airways. TGF-β1 is expressed in alveolar macrophages and airway epithelial cells of COPD patients [165] and is released from epithelial cells of small airways [20]. TGF-β is released in a latent from and activated by various factors,

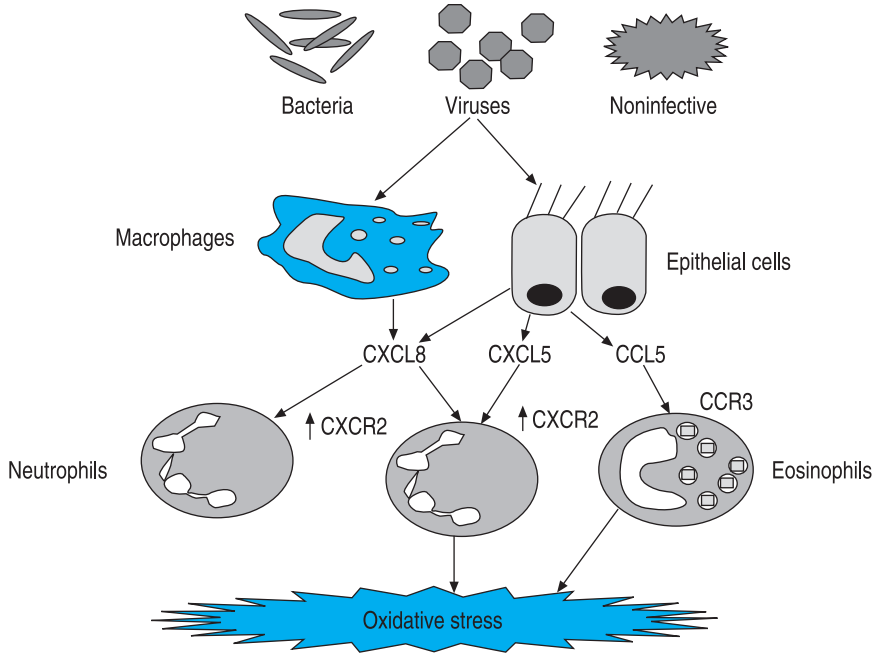

Fig. 6. – Chemokines in exacerbations of chronic obstructive pulmonary disease. CXCL8 is released from macrophages and epithelial cells in response to infective agents or environmental stimuli and act on CXCR2, which are upregulated during exacerbations. CXCL5 is also released from epithelial cells to act on the same receptors. These cells also express CCL5, which may act on CCR3 leading to attraction of eosinophils.

including MMP-9 [166]. It may play an important role in the characteristic peribronchiolar fibrosis of small airways, either directly or through the release of connective tissue growth factor (fig. 7). TGF-β potently downregulates β_2-adrenergic receptors by inhibiting gene transcription in human cell lines [167] and markedly reduces the bronchodilator response to β-agonists in airway smooth muscle *in vitro* [168]. Alveolar macrophages produce TGF-α in much greater amounts than TGF-β [169] and this may be a major endogenous activator of EGFR, which plays a key role in regulating mucus secretion in response to many stimuli, including cigarette smoke. Cigarette smoke activates TNF-α converting enzyme (TACE) on airway epithelial cells, which results in the shedding of TGF-α and the activation of EGFR, resulting in increased mucus secretion [170]. The mucus secretory response to cigarette smoke is inhibited by knock-down of TGF-α and TACE by interference RNA. Epidermal growth factor also activates EGFR, which mediates increased secretion of mucus and expression of mucin genes in response to oxidative stress and cigarette smoke (fig. 8) [171].

VEGF is a major regulator of vascular growth and is likely to be involved in the pulmonary vascular remodeling that occurs as a result of hypoxic pulmonary vasoconstriction in severe COPD [172]. There is increased expression of VEGF in pulmonary vascular smooth muscle of patients with mild and moderate COPD but, paradoxically, a reduction in expression in severe COPD with emphysema [110]. Inhibition of VEGF receptors in rats using a selective inhibitor induces apoptosis of alveolar endothelial cells resulting in emphysema [21] and this appears to be associated with oxidative stress [173]. Interestingly, the concentration of VEGF is increased in

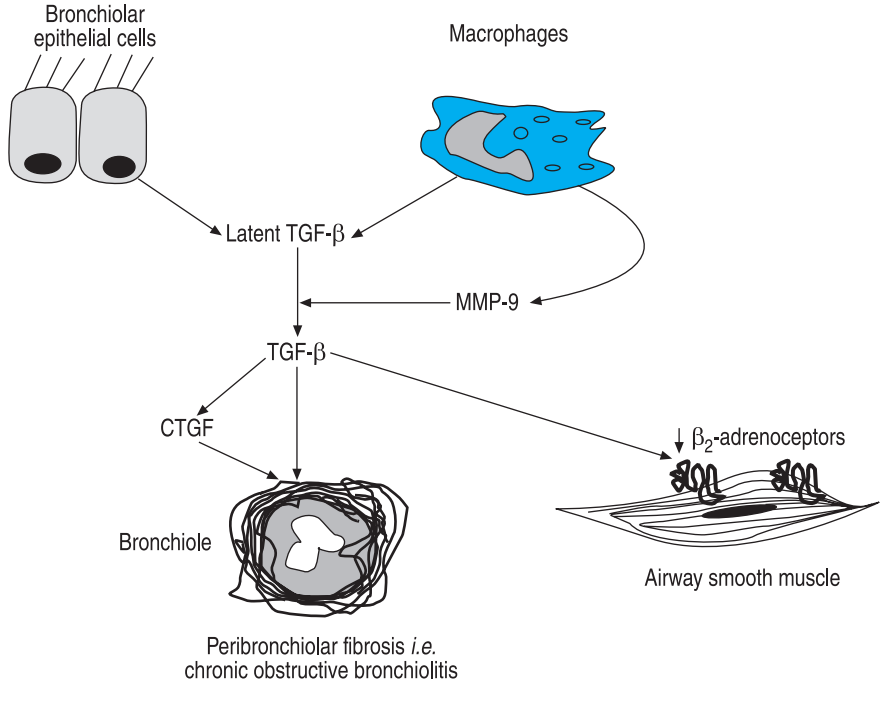

Fig. 7. – Transforming growth factor (TGF)-β in chronic obstructive pulmonary disease. TGF-β is released in a latent form that may be activated by matrix metalloproteinase (MMP)-9. It may then cause fibrosis directly through effects on fibroblasts or indirectly *via* the release of connective tissue growth factor (CTGF). TGF-β may also downregulate β_2-adrenoceptors on cells, such as airway smooth muscle, to diminish the bronchodilator response to β-agonists.

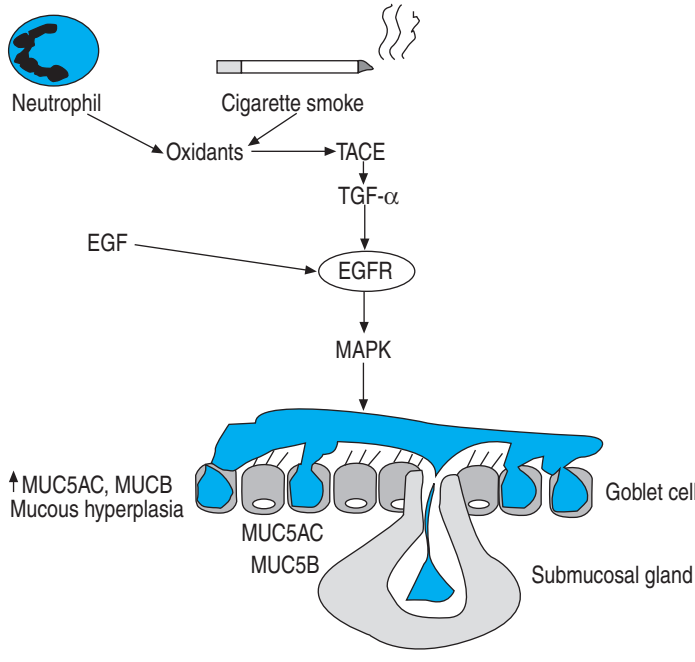

Fig. 8. – Epidermal growth factor receptors (EGFR) in chronic obstructive pulmonary disease. EGFR play a key role in the regulation of mucus hypersecretion, with increased expression of mucin genes (MUC5AC, MUCB) and differentiation of goblet cells, as well as hyperplasia of mucus-secreting cells. These effects are mediated *via* the activation of mitogen-activated protein kinases (MAPK). EGFR are activated by transforming growth factor (TGF)-α, which in turn is activated by tumour necrosis factor-α converting enzyme (TACE), activated *via* the release of oxidants from cigarette smoke and neutrophils. EGFR may also be activated by epidermal growth factor (EGF).

induced sputum of patients with asthma and chronic bronchitis, but is significantly reduced in COPD patients with emphysema [174, 175]. In addition, VEGF is also an important pro-inflammatory cytokine produced by epithelial and endothelial cells, macrophages and activated T-cells, which acts by increasing endothelial cell permeability, by inducing expression of endothelial adhesion molecules and *via* its ability to act as a monocyte chemoattractant; it also stimulates DCs. Among the several chemokine–chemokine receptors induced by VEGF, CXCL10 and its receptor CXCR3 might be the most important. Thus, VEGF may be an intermediary between cell-mediated immune inflammation and the associated angiogenesis reaction [176, 177].

Conclusions

In summary, cigarette-smoke exposure induces a florid inflammatory response in the lung involving structural and inflammatory cells and a large array of inflammatory mediators (fig. 9). The interaction of these complex steps eventually leads to airway remodelling and obstruction and emphysema, albeit in only 20% of chronic smokers. Interestingly, the main difference between smokers who develop COPD and those who do not seems to be the presence of an adaptive immune response with CD8+, CD4+ and B-cells that express obvious signs of being activated effector cells. Moreover, the main difference between resistant and susceptible smokers in an animal model of emphysema

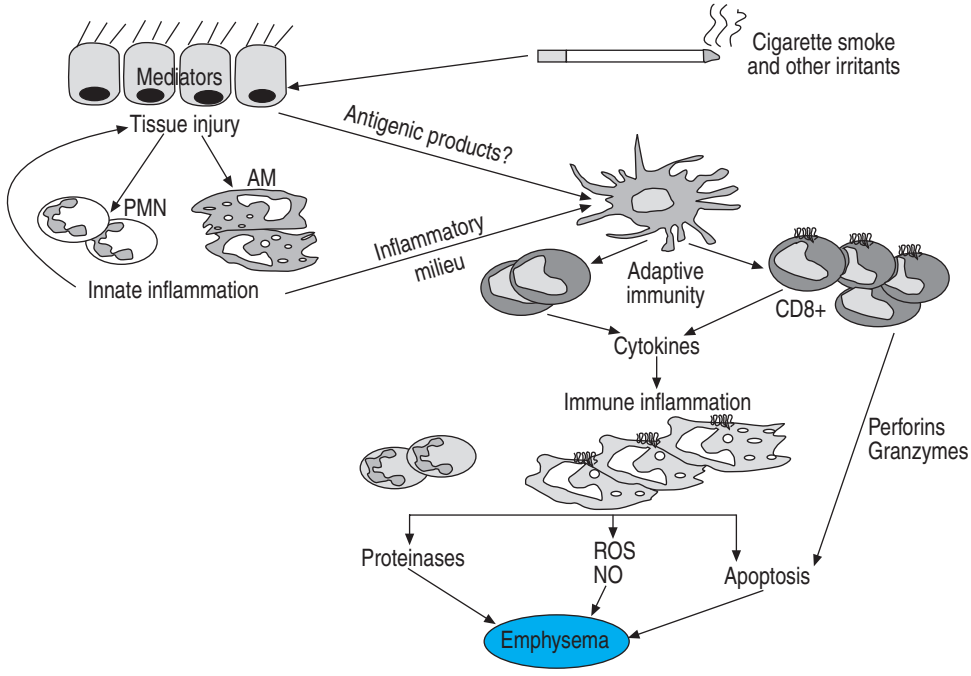

Fig. 9. – Inflammatory mechanisms in chronic obstructive pulmonary disease (COPD). The epithelium reacts to cigarette smoke by promoting an innate inflammatory reaction, which damages lung cells and interstitium. Damaged tissue can become antigenic and be presented to dendritic cells in pulmonary lymphatics. The innate inflammatory response creates a propitious microenvironment for dendritic cell maturation and cross-presentation of antigens to CD4+ and CD8+ T-cells. Once activated, T-cells will proliferate CD8+ cells in larger numbers than CD4+ cells and migrate to the lung under the direction of T-helper (Th)1 chemokines. Activated T-cells in the lung produce Th1 cytokines and other mediators, which induce an "immune inflammation" with innate immune cells. These cells are activated to produce proteinases, oxidative radicals and inflammatory mediators, which, along with apoptosis and cell necrosis, would produce the airway and perenchymal changes in COPD. PMN: polymorphonuclear cell; AM: alveolar macrophage; ROS: reactive oxygen species; NO: nitric oxide.

secondary to cigarette-smoke exposure, is the presence of an adaptive immune cell response comprising CD8+ and CD4+ T-cells and associated cytokines and chemokines similar to human smokers.

It is likely that genetic and epigenetic factors are involved in determining the progression of the inflammatory cascade, as this is supported by animal models that look at different strains. Mice strains resistant to cigarette smoke-induced emphysema have a genetic response to smoke exposure that decreases the expression of multiple inflammatory genes (many similar to the ones seen in humans) and increases the expression of anti-inflammatory genes, which effectively prevents inflammation and likely emphysema. Genetically different susceptible strains react in an opposite manner increasing the expression of inflammatory genes both of the innate and adaptive immunity [178].

Which of the cells or inflammatory mediators described here are responsible for the progression of the disease in smokers? Probably all acting together as redundant and obligatory players in a complex innate and adaptive immune response, and probably not a single one in particular, which makes selection for therapeutic goals very difficult. The future comprehension of COPD would surely be the understanding of which genes or gene master switch orchestrate the progression of inflammation towards the full disease.

Summary

Many inflammatory cells and mediators have been implicated in the pathogenesis of chronic obstructive pulmonary disease. There are increased numbers of macrophages, neutrophils and T-lymphocytes (particularly CD8+ cells), and the release of multiple inflammatory mediators (lipids, chemokines, cytokines, growth factors). Macrophages appear to play an important role in orchestrating the inflammatory process, including the recruitment of neutrophils and T-cells into small airways and lung parenchyma. A high level of oxidative and nitrative stress may amplify this inflammation.

Keywords: Cytokine, dendritic cell, macrophage, neutrophil, oxidative stress, T-lymphocyte.

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