Cellular and Molecular Mechanisms of Chronic Obstructive Pulmonary Disease

Peter J. Barnes, FRS, FMedSci

KEYWORDS

• Inflammation • Macrophage • Neutrophil • Oxidative stress • Cytokine • Chemokine • Autoantibody • Nuclear factor-κB

KEY POINTS

- Chronic inflammation in peripheral airways and lung parenchyma in patients with chronic obstructive pulmonary disease (COPD) may underlie progressive airways obstruction, may flare up during infective exacerbations, and extend into the systemic circulation to contribute to comorbidities.
- Several cells are involved in COPD inflammation, including macrophages, epithelial cells, dendritic cells, neutrophils, eosinophils, and T and B lymphocytes.
- These inflammatory and structural cells release many inflammatory mediators that contribute to the pathophysiology of COPD, including lipid mediators, cytokines, chemokines, and growth factors.
- Oxidative stress, including defective antioxidant defenses, plays a key role in the mechanisms of COPD, with activation of inflammatory genes, cellular senescence, autoimmunity, and corticosteroid resistance.
- There are several abnormal disease processes in COPD that may reveal novel therapeutic targets, including accelerated aging, defective phagocytosis, and failure to resolve inflammation and repair.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) involves chronic inflammation of the lung, particularly in peripheral airways and parenchyma, which increases during acute exacerbations. It is also associated with systemic inflammation, which may contribute to or worsen several comorbidities and may be derived from overspill from the peripheral lung.[1] It is important to understand the nature of this inflammatory response in order to develop effective antiinflammatory treatments for COPD in the future.[2]

COPD is an obstructive disease of the lungs that slowly progresses over many decades, leading to death from respiratory failure unless patients die of comorbidities, such as cardiovascular disease and lung cancer, before this stage. Although the commonest cause of COPD is chronic cigarette smoking, some patients, particularly in developing countries, develop the disease from inhalation of smoke from burning biomass fuels or other inhaled irritants.[3] However, only about 25% of smokers develop COPD, suggesting that there may be genetic, epigenetic, or host factors that predispose to its development, although these have not yet been identified.

PATHOLOGY

The progressive airflow limitation in COPD is caused by 2 major pathologic processes: remodeling and narrowing of small airways and destruction of the lung parenchyma with consequent destruction of the alveolar attachments of these airways as a result of emphysema. These

National Heart and Lung Institute, Imperial College, Dovehouse Street, London SW3 6LY, UK
E-mail address: p.j.barnes@imperial.ac.uk

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pathologic changes are caused by chronic inflammation in the lung periphery, which increases as the disease progresses.[4] Even in mild disease there is obstruction and loss of small airways.[5] Analysis of serial computed tomography scans suggests that small airway obstruction usually precedes the development of emphysema.[6] The small airway obstruction and loss of alveolar attachments result in airway closure and air trapping and hyperinflation. These changes worsen on exercise, resulting in exertional dyspnea, the major symptom of COPD.

COPD AS AN INFLAMMATORY DISEASE

There is a characteristic pattern of inflammation with increased numbers of macrophages, T lymphocytes, and B lymphocytes, together with increased numbers of neutrophils in the lumen.[7–9] The inflammatory response in COPD involves both innate and adaptive immune responses, which are linked through the activation of dendritic cells.[10] Multiple inflammatory mediators derived from inflammatory cells and structural cells of the airways and lungs are increased in COPD.[11] A similar pattern of inflammation and mediator expression is seen in smokers without airflow limitation, but in COPD this inflammation seems to be amplified and further increased during acute exacerbations precipitated by bacterial or viral infection. The molecular basis for the amplification of inflammation is not yet fully understood but may be, at least in part, determined by genetic and epigenetic factors. Cigarette smoke and other irritants inhaled into the respiratory tract may activate surface macrophages and airway epithelial cells to release multiple chemotactic mediators, particularly chemokines, which attract circulating neutrophils, monocytes, and lymphocytes into the lungs.[12] This inflammation persists even when smoking is stopped, suggesting that there are self-perpetuating mechanism, although these have not yet been elucidated.[13] It is possible that memory T cells, bacterial colonization, or autoimmunity may drive the persistent inflammation in patients with COPD.

INFLAMMATORY CELLS

The inflammation of COPD lungs involves both innate immunity (neutrophils, macrophages, eosinophils, mast cells, natural killer cells, gamma delta T cells, and dendritic cells) and adaptive immunity (T and B lymphocytes) but also involves the activation of structural cells, including airway and alveolar epithelial cells, endothelial cells, and fibroblasts.

EPITHELIAL CELLS

Epithelial cells are activated by cigarette smoke and other inhaled irritants, such as biomass fuel smoke, to produce inflammatory mediators, including tumor necrosis factor (TNF) alpha, interleukin (IL)-1 beta, IL-6, granulocyte-macrophage colony–stimulating factor (GM-CSF), and CXCL8 (IL-8). Epithelial cells in small airways may also be an important source of transforming growth factor (TGF) beta which then induces local fibrosis. Vascular endothelial growth factor (VEGF) seems to be necessary to maintain alveolar cell integrity, and blockade of VEGF receptors (VEGFR2) in rats induces apoptosis of alveolar cells and an emphysemalike disorder.[14] Airway epithelial cells are also important in defense of the airways, with mucus production from goblet cells, and secretion of antioxidants, antiproteases, and defensins. It is possible that cigarette smoke and other noxious agents may impair these responses of the airway epithelium, increasing susceptibility to infection. The airway epithelium in chronic bronchitis and COPD often shows squamous metaplasia, which may result from increased proliferation of basal airway epithelial cells but the nature of the growth factors involved in epithelial cell proliferation, cell cycle, and differentiation in COPD are not yet certain. Epithelial growth factor receptors (EGFR) show increased expression in airway epithelial cells of patients with COPD and may contribute to basal cell proliferation, resulting in squamous metaplasia and an increased risk of bronchial carcinoma.[15]

MACROPHAGES

Macrophages play a key role in the pathophysiology of COPD and may orchestrate the chronic inflammatory response (**Fig. 1**).[16] There is a marked increase (5-fold to 10-fold) in the numbers of macrophages in airways, lung parenchyma, bronchoalveolar lavage (BAL) fluid, and sputum in patients with COPD. Macrophages are localized to sites of alveolar wall destruction in patients with emphysema and there is a correlation between macrophage numbers in the parenchyma and severity of emphysema.[17] Macrophages may be activated by cigarette smoke extract to release inflammatory mediators, including TNF-α, CXCL1, CXCL8, CCL2 (MCP-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.[18] Alveolar macrophages from

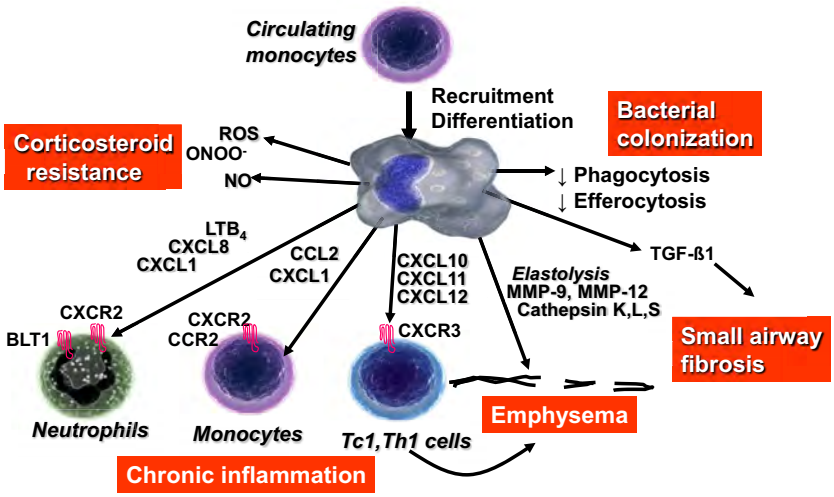

Fig. 1. Central role of alveolar macrophages in COPD. Alveolar macrophages are derived from circulating monocytes that differentiate within the lung. They secrete many inflammatory proteins that may orchestrate the inflammatory process in COPD. Neutrophils may be attracted by CXCL8, CXCL1, and leukotriene B_4 (LTB_4); monocytes by CCL2, and Tc1; and Th1 lymphocytes by CXCL10, CXC11, and CXCL12. Release of elastolytic enzymes including matrix metalloproteinases (MMP) and cathepsins causes elastolysis, which contributes to emphysema together with cytotoxic T cells. Release of TGF-β1 may induce fibrosis of small airways. Macrophages generate reactive oxygen species (ROS) and nitric oxide (NO), which together form peroxynitrite ($ONOO^-$) and may contribute to corticosteroid resistance. Defective bacterial phagocytosis may lead to bacterial colonization.

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.[18] Macrophages show this difference even when maintained in culture for 3 days and therefore seem to be intrinsically different from the macrophages of normal smokers and nonsmoking normal control subjects.[18]

There may be different phenotypes of macrophage that may be differently activated and with different responses. The murine M1 of classically activated macrophages are proinflammatory, whereas M2 or alternatively activated macrophages are more antiinflammatory, release IL-10, and show marked phagocytic activity.[19] However, these distinctions are less clear in human macrophages and the surface markers of these phenotypes less distinct. In general, it is likely that M1-like macrophages predominate in COPD, but further studies are needed.

The predominant elastolytic enzyme secreted by alveolar macrophages in patients with COPD is MMP-9. Most of the inflammatory proteins that are upregulated in COPD macrophages are regulated by the transcription factor nuclear factor kappa B (NF-κB), which is activated in alveolar macrophages of patients with COPD, particularly during exacerbations.[20] The increased numbers of macrophages in the lungs of smokers and patients with COPD are caused by increased recruitment of monocytes from the circulation in response to the monocyte-selective chemokines CCL2 and CXCL1, which are increased in sputum and BAL of patients with COPD.[21] Monocytes from patients with COPD show a greater chemotactic response to CXCL1 than cells from normal smokers and nonsmokers, but this is not explained by an increase in its receptor CXCR2.[22] Although all monocytes express CCR2, the receptor for CCL2, only ~30% of monocytes express CXCR2. Macrophages also release CXCL9, CXCL10, and CXCL11, which are chemotactic for $CD8^+$ Tc1 and $CD4^+$ Th1 cells, via interaction with the chemokine receptor CXCR3 expressed on these cells.[23] Macrophages from patients with COPD release more inflammatory proteins than macrophages from normal smokers and nonsmokers, indicating increased activation.[24]

Corticosteroids are ineffective in suppressing inflammation, including cytokines, chemokines, and proteases, in patients with COPD.[25] 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.[24] The reasons for resistance to corticosteroids in COPD may be the marked reduction in activity of histone deacetylase (HDAC) 2,[26,27] which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes. The reduction in

HDAC activity in macrophages is correlated with increased secretion of cytokines like TNF-α and CXCL8, and reduced response to corticosteroids. The reduction of HDAC2 activity in patients with COPD may be mediated through oxidative stress and peroxynitrite formation.[28]

Both alveolar macrophages and monocyte-derived macrophages from patients with COPD also show reduced phagocytic uptake of bacteria and this may be a factor in determining chronic colonization of the lower airways by bacteria such as *Haemophilus influenzae* or *Streptococcus pneumoniae*.[29] COPD macrophages are also defective in taking up apoptotic cells and this may contribute to the failure to resolve inflammation in COPD.[30] The nature of this defect in phagocytosis is not fully understood, but it seems to be caused by a defect in microtubular function that is required for phagocytosis rather than any abnormality of recognition of the phagocytosed particles.[31] The bacterial colonization of lower airways may predispose to increased acute exacerbations and also to the increased risk of developing community-acquired pneumonia in patients with COPD.[32]

NEUTROPHILS

Increased numbers of activated neutrophils are found in sputum and BAL fluid of patients with COPD,[33] although few neutrophils are seen airway wall and lung parenchyma, likely reflecting their rapid transit through these tissues. Neutrophil numbers in induced sputum correlate with COPD disease severity.[33] 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 (G-CSF) released from lung macrophages. Neutrophil recruitment to the airways and parenchyma involves initial adhesion to endothelial cells via E-selectin, which is upregulated on endothelial cells in the airways of patients with COPD. Adherent neutrophils migrate into the respiratory tract under the direction of various neutrophil chemotactic factors, including LTB_4, CXCL1, CXCL5 (ENA-78), and CXCL8, which are increased in COPD airways.[21] These chemotactic mediators may be derived from alveolar macrophages, T cells, and epithelial cells, but the neutrophil may be a major source of CXCL8. Neutrophils recruited to the airways of patients with COPD are activated because there are increased concentrations of granule proteins, such as myeloperoxidase (MPO), and human neutrophil lipocalin, in the sputum supernatant.[34] 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. Airway neutrophilia is linked to mucus hypersecretion because neutrophil elastase, cathepsin G, and proteinase-3 are potent stimulants of mucus secretion from submucosal glands and goblet cells. There is a marked increase in neutrophil numbers in the airways in acute exacerbations of COPD accounting for the increased purulence of sputum, which may reflect increased production of neutrophil chemotactic factors, including LTB_4 and CXCL8.[35,36] Neutrophils from patients with COPD show marked abnormalities in chemotactic response, with increased migration but reduced accuracy,[37] reminiscent of the abnormal monocyte chemotactic responses.[38]

EOSINOPHILS

Although eosinophils are the predominant leukocyte in asthma, their role in COPD is less certain. Increased numbers of eosinophils have been described in the airways and BAL of patients with stable COPD, whereas other investigators have not found increased numbers in airway biopsies, BAL, or induced sputum.[39] The presence of eosinophils in patients with COPD predicts a response to corticosteroids and may indicate coexisting asthma.[40,41] Increased numbers of eosinophils have been reported in bronchial biopsies and BAL fluid during acute exacerbations of chronic bronchitis.[42–44] The levels of eosinophil basic proteins in induced sputum are as increased in COPD as in asthma, despite the absence of eosinophils, suggesting that they may have degranulated and are no longer recognizable by microscopy.[34] Perhaps this is caused by the high levels of neutrophil elastase that have been shown to cause degranulation of eosinophils.[45]

DENDRITIC CELLS

Dendritic cell plays a central role in the linking of the innate to the adaptive immune response. The airways and lungs contain a rich network of dendritic cells that are localized near the surface, so that they are ideally located to signal the entry of foreign substances that are inhaled. Dendritic cells can activate a variety of other inflammatory and immune cells, including macrophages, neutrophils, and T and B lymphocytes, so dendritic cells may play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents. Dendritic cells seem to be activated in the lungs of patients with COPD[46] and are linked to disease severity.[47]

T LYMPHOCYTES

There is an increase in the total numbers of T lymphocytes in lung parenchyma, peripheral airways, and central airways of patients with COPD, with the greater increase in $CD8^+$ than $CD4^+$ cells.[4,23] There is a correlation between the numbers 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 $CD8^+$ (Tc1), in patients with COPD. There is also an increase in the absolute number of $CD4^+$ (Th1) T cells, albeit in smaller numbers, in the airways of smokers with COPD and these cells express activated STAT-4, a transcription factor that is essential for activation and commitment of the Th1 lineage.[48] $CD4^+$ Th17 cells, which secrete IL-17A and IL-22, are also increased in airways of patients with COPD and may play a role in orchestrating neutrophilic inflammation.[49,50] Th17 cells may be regulated by IL-6 and IL-23 released from alveolar macrophages. $CD4^+$ and $CD8^+$ T cells in the lungs of patients with COPD show increased expression of CXCR3, a receptor activated by the chemokines CXCL9, CXCL10, and CXCL11, all of which are increased in COPD.[51] 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.[52] $CD8^+$ cells are typically increased in airway infections and it is possible that the chronic bacterial colonization of the lower respiratory tract of patients with COPD is responsible for this inflammatory response.

Autoimmune mechanisms may also be involved. Cigarette-induce lung injury may uncover previously sequestered autoantigens, or cigarette smoke may damage lung interstitial and structural cells and make them antigenic.[8] Oxidative stress may result in the formation of carbonylated proteins that are antigenic, and several anticarbonylated protein antibodies have been found in the circulation of patients with COPD, particularly in severe disease.[53] Antiendothelial antibodies have also been detected.[53,54] Autoantibodies may cause cell damage through the binding of complement, which is increased in the lungs of patients with COPD.

$CD8^+$ cells cause cytolysis and apoptosis of alveolar epithelial cells through release of perforins, granzyme B, and TNF-α, and there is an association between $CD8^+$ cells and apoptosis of alveolar cells in emphysema.[55] There is evidence for immunologic senescence in COPD with increased numbers of T cells with no expression of the costimulatory receptor CD28 ($CD4/CD28^{null}$, $CD8/CD28^{null}$ cells) and these cells release increased amounts of perforins and granzyme B.[56,57]

MEDIATORS OF INFLAMMATION

Many inflammatory mediators have now been implicated in COPD, including lipids, free radicals, cytokines, chemokines, and growth factors.[11] These mediators are derived from inflammatory and structural cells in the lung and interact with each other in a complex manner. Because so many mediators are involved, it is unlikely that blocking a single mediator will have much clinical impact. Similar mediators in the lungs of patients with COPD may also be increased in the circulation and this systemic inflammation may underlie and potentiate comorbidities, as discussed later.

Lipid Mediators

The profile of lipid mediators in exhaled breath condensates of patients with COPD shows an increase in prostaglandins and leukotrienes.[58] There is a significant increase in prostaglandin E_2 and prostaglandin $F_{2\alpha}$ and an increase in LTB_4 but not cysteinyl-leukotrienes. This pattern is different from that seen in asthma, in which increases in thromboxane and cysteinyl-leukotrienes have been shown. The increased production of prostanoids in COPD is likely to be secondary to the induction of cyclo-oxygenase-2 (COX2) by inflammatory cytokines, and increased expression of COX2 is described in alveolar macrophages of patients with COPD. LTB_4 concentrations are also increased in induced sputum and further increased in sputum and exhaled breath condensate during acute exacerbations.[35] 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 approximately 25%.[59] BLT_1-receptors were recently identified on T lymphocytes and there is evidence that LTB_4 is also involved in recruitment of T cells.

Cytokines

Cytokines are the mediators of chronic inflammation and several have been implicated in COPD.[60,61] There is an increase in concentration of TNF-α in induced sputum in stable COPD with a further increase during exacerbations.[33,36] TNF-α production from peripheral blood monocytes is also increased in patients with COPD and has been implicated in the cachexia and skeletal muscle apoptosis in some patients with

severe disease. TNF-α is a potent activator of NF-κB and this may amplify the inflammatory response. However, anti-TNF therapies have not proved to be effective in patients with COPD and may have serious adverse effects. IL-1β and IL-6 are other proinflammatory cytokines that may amplify the inflammation in COPD and may be important for systemic circulation. IL-1β and the related cytokine IL-18 may be produced via the activation of the NLRP3 inflammasome by cellular stress, including bacterial infections.[62] IL-17, and other Th17 cytokines, are also increased in COPD sputum and airways and may play a role in orchestrating neutrophilic inflammation in the lungs.[49,63]

Chemokines

Several chemokines have been implicated in COPD and have been of particular interest because chemokine receptors are G protein–coupled receptors, for which small molecule receptor antagonists have been developed.[12] CXCL8 concentrations are increased in induced sputum of patients with COPD and increase further during exacerbations.[33,36] 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 increased in sputum and BAL fluid of patients with COPD and this chemokine may be more important as a chemoattractant than CXCL8, acting via CXCR2, which are expressed on neutrophils and monocytes.[21] CXCL1 induces significantly more chemotaxis of monocytes of patients with COPD compared with those of normal smokers and this may reflect increased turnover and recovery of CXCR2 in monocytes of patients with COPD.[22] 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.

CCL2 is increased in concentration in COPD sputum and BAL fluid[21] and plays a role in monocyte chemotaxis via activation of CCR2. CCL2 seems to cooperate with CXCL1 in recruiting monocytes into the lungs. The chemokine CCL5 (RANTES) is also expressed in airways of patients with COPD during exacerbations and activates CCR5 on T cells and CCR3 on eosinophils, which may account for the increased eosinophils and T cells in the walls of large airways that have been reported during exacerbations of chronic bronchitis. As discussed earlier, CXCR3 are upregulated on Tc1 and Th1 cells of patients with COPD with increased expression of their ligands CXCL9, CXCL10, and CXCL11.[51]

Growth Factors

TGF-β1 is expressed in alveolar macrophages and airway epithelial cells of patients with COPD and is released from epithelial cells of small airways. TGF-β is released in a latent from and activated by various factors, including MMP-9 and oxidative stress. TGF-β may play an important role in the characteristic peribronchiolar fibrosis of small airways, either directly or through the release of connective tissue growth factor. Alveolar macrophages produce TGF-α in greater amounts than TGF-β and this may be a major endogenous activator of EGFR that play a key role in regulating mucus secretion in response to many stimuli, including cigarette smoke. Cigarette smoke activates TNF-α-converting enzyme on airway epithelial cells, which results in the shedding of TGF-α and the activation of EGFR, resulting in increased mucus secretion.[64]

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. 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. Inhibition of VEGF receptors using a selective inhibitor induces apoptosis of alveolar endothelial cells in rats, resulting in emphysema, and this seems to be driven by oxidative stress.[65]

PROTEASES

The increase in elastase activity in patients with COPD may contribute to the development of emphysema and to neutrophilic inflammation through the generation of chemotactic peptides such as Pro-Gly-Pro (matrikines). Human neutrophil elastase not only has elastolytic activity but is also a potent stimulant of mucus secretion in the airways. MMP9 seems to be the predominant elastolytic enzyme in COPD and is secreted from macrophages, neutrophils, and epithelial cells. MMP9 causes elastolysis but also stimulates neutrophilic inflammation through the generation of N-acetyl-PGP (Proline-Glycine-Proline).[66] MMP9 activation has been implicated in skin wrinkling and in arterial stiffness, which are indicators of COPD comorbidity.

OXIDATIVE STRESS

Oxidative stress occurs when ROS are produced in excess of the antioxidant defense mechanisms and result in harmful effects, including damage to lipids, proteins, and DNA. Oxidative stress is a critical feature in COPD.[67] Inflammatory and structural cells, including neutrophils, macrophages, and epithelial cells that are activated in the airways of patients with COPD, produce ROS. Superoxide anions (O_2^-) are generated by nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase and converted to hydrogen peroxide (H_2O_2) by superoxide dismutases. H_2O_2 is then converted to water by catalase. O_2^- and H_2O_2 may interact in the presence of free iron to form the highly reactive hydroxyl radical (OH). O_2^- may also combine with NO to form peroxynitrite, which also generates OH. 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, including bronchoconstriction and plasma exudation.[68] Peroxynitrite is also increased in the breath of patients with COPD.[69] Nitric oxide may be increased in peripheral lung of patients with COPD and seems to be linked to increased expression of inducible and neural NO synthases (NOS2, NOS1).[69,70]

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract, including catalase, superoxide dismutase (SOD), and glutathione, formed by the enzyme gamma-glutamyl cysteine ligase, and glutathione synthetase. In the lung, intracellular antioxidants are expressed at low levels and are not induced by oxidative stress, whereas the major antioxidants are extracellular. Extracellular antioxidants, particularly glutathione peroxidase, are markedly upregulated in response to cigarette smoke and oxidative stress. Extracellular antioxidants also include the dietary antioxidants vitamin C (ascorbic acid) and vitamin E (alpha-tocopherol), uric acid, lactoferrin, and extracellular SOD3, which is highly expressed in human lung. Most antioxidants are regulated by the transcription factor nuclear erythroid-2–related factor-2 (Nrf2), which is activated by oxidative stress. However, in COPD lungs and cells, Nrf2 is not appropriately activated despite high levels of oxidative stress in the lungs[71,72] and this may be related to increased acetylation caused by decreased HDAC2.[73]

ROS have wide-ranging effects on the airways and parenchyma and increase the inflammatory response (**Fig. 2**). ROS activate NF-κB, which switches on multiple inflammatory genes resulting in amplification of the inflammatory response. 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.[74] Oxidative stress may also impair the function of antiproteases such as alpha$_1$-antitrypsin and SLPI, and thereby accelerates the breakdown of elastin in lung parenchyma. Oxidative stress markedly reduces HDAC2 activity and expression, through activation of phosphoinositide-3-kinase-delta (PI3Kδ) and

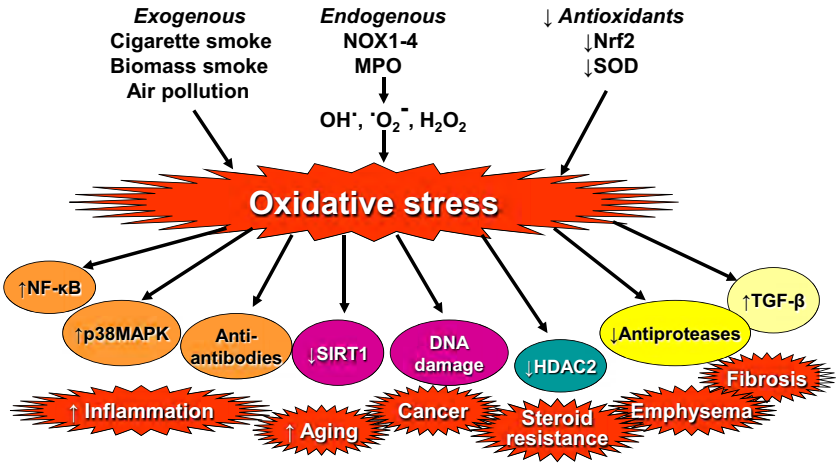

Fig. 2. Oxidative stress in COPD. Oxidative stress may be increased in COPD by a reduction in the transcription factor Nrf2, activation of NADPH oxidases (NOX), MPO, and reduced superoxide dismutase (SOD). Oxidative stress is a key driving mechanisms in COPD through activation of the proinflammatory transcription factor nuclear factor-KB (NF-κB), p38 mitogen-activated protein kinase (MAPK), generation of autoantibodies to carbonylated proteins, reduced sirtuin-1 (SIRT1), DNA damage, reduced histone deacetylase (HDAC)-2, reduced antiproteases, and increased TGF-β.

peroxynitrite-induced nitration of tyrosine resides. This process amplifies inflammation further and prevents corticosteroids from inactivating activated inflammatory genes. Through similar mechanisms, oxidative stress reduces the expression and activity of sirtuin-1, a key repair molecule that is implicated in aging. The reduction in sirtuin-1 in COPD lungs and cells may underlie the accelerated aging response seen in COPD.[75] Oxidative stress may also predispose to lung cancer, through the activation of growth factors and via DNA damage.[76] Oxidative stress leads to formation of carbonylated proteins, which may be antigenic and stimulate the development of autoantibodies in patients with COPD.[53]

There is also evidence for systemic oxidative stress in patients with COPD with an increase in lipid peroxidation products and decreased antioxidant capacity. Furthermore, circulating neutrophils release more ROS than neutrophils from normal smokers and nonsmokers.[77] Systemic oxidative stress increases further during acute exacerbations.[78]

SYSTEMIC INFLAMMATION IN COPD

Patients with COPD, particularly when the disease is severe and during exacerbations, have evidence of systemic inflammation, measured either as increased circulating cytokines, chemokines, and acute phase proteins, or as abnormalities in circulating cells (**Fig. 3**).[79,80] Smoking may cause systemic inflammation (for example, increased total leukocyte count) but in patients with COPD the degree of systemic inflammation is greater. It is still uncertain whether these systemic markers of inflammation are a spill-over from inflammation in the peripheral lung, are a parallel abnormality, or are related to some comorbid disease that then has effects on the lung. In any case, the components of this systemic inflammation may account for the systemic manifestations of COPD and may worsen comorbid diseases. In a large population study, systemic inflammation (increased C-reactive protein [CRP], fibrinogen, and leukocytes) was associated with a 2-fold to 4-fold increased risk of cardiovascular disease, diabetes, lung cancer, and pneumonia, but not with depression.[81] Using 6 inflammatory markers (CRP, IL-6, CXCL8, fibrinogen, TNF-α, and leukocytes), 70% of patients with COPD had some components of systemic inflammation and 16% had persistent inflammation.[82] Patients with persistent systemic inflammation had increased mortality and more frequent exacerbations. Systemic inflammation seems to relate to accelerated decline in lung function and is increased further during exacerbations.[83]

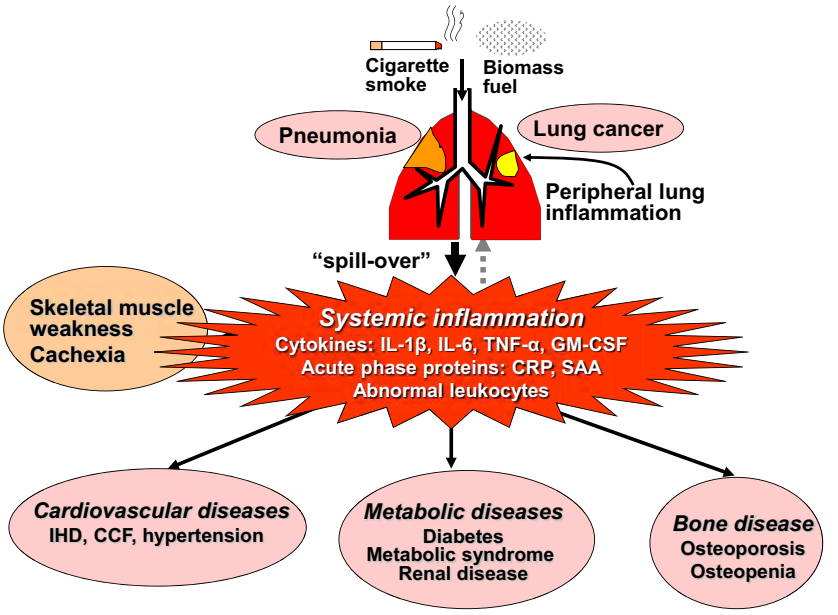

Fig. 3. Systemic inflammation and comorbidities in COPD. Patients with COPD have peripheral lung inflammation that may extend into the systemic circulation, leading to skeletal muscle weakness and cachexia and increasing propensity to cardiovascular, metabolic, and bone diseases. There is an increase in circulating cytokines, including IL-1β, IL-6, TNF-α, and GM-CSF, as well as acute phase proteins, such as C-reactive protein (CRP), serum amyloid A (SAA), and abnormal leukocytes. Peripheral lung inflammation may also increase the risk of developing lung cancer and community-acquired pneumonia. CCF, congestive cardiac failure; IHD, ischemic heart disease.

Acute Phase Proteins

CRP is an acute phase protein that is increased in plasma of patients with COPD, particularly during acute infective exacerbations. In stable COPD, plasma concentrations are related to all-cause mortality in mild to moderate patients,[84] but not in severe or very severe patients.[85] However, although CRP is related to forced expiratory volume in 1 second (FEV_1) in cross-sectional studies, there is no association with progressive decline of FEV_1 in longitudinal studies.[86] CRP is also increased in exacerbations of COPD, whether because of viral or bacterial causes,[83] and a high concentration of CRP 2 weeks after an exacerbation predicts the likelihood of recurrent exacerbation.[87] CRP in plasma is produced by the liver in response to circulating IL-6 and therefore may be a biomarker of systemic inflammation rather than directly contributing to comorbid diseases.

Plasma fibrinogen concentrations are increased in patients with COPD with frequent exacerbations.[78,88] An increased plasma fibrinogen in a population is related to worse FEV_1 and increased risks of hospitalization for COPD.[89] Serum amyloid A (SAA) is another acute phase protein released by circulating proinflammatory cytokines from liver, but, unlike CRP, also from inflamed tissue. It has been identified by proteomic analysis of plasma as showing an increase during acute exacerbations of COPD and its concentrations are correlated with the severity of exacerbations.[90] SAA binds to gram-negative bacteria and is part of the innate defense mechanism against bacterial infections, but it also has proinflammatory effects, including activation of epithelial cells, neutrophils, monocytes, and Th17 cells to release proinflammatory mediators and inhibit the effects of antiinflammatory lipoxins.[91] SAA activates Toll-like receptor (TLR) 2, resulting in activation of NF-κB.[92] SAA also binds to the receptor for advanced glycation end products (RAGE) and soluble RAGE concentrations are reduced in plasma of patients with COPD and are related to disease severity.[93]

Surfactant protein-D (SP-D) is a glycoprotein member of the collectin family and is secreted mainly by type II pneumocytes and Clara cells and plays a role in innate defense against microorganisms. Serum SP-D concentrations are increased in smokers and in patients with COPD but are weakly correlated with disease severity.[94] Serum SP-D is further increased transiently during exacerbations. Serum SP-D is also increased in other lung diseases, including asthma, pulmonary fibrosis, and pneumonia, and so has poor specificity. Although SP-D in serum is increased in COPD, its concentrations are reduced in BAL fluid, suggesting that it may translocate from lung to systemic circulation.[95] The reduced concentration of SP-D in BAL fluid may be caused by oxidative damage of the quaternary structure, so it is not detectable by immunoassay.

Cytokines

IL-6 is consistently increased in the systemic circulation of patients with COPD, particularly during exacerbations, and may account for the increase in circulating acute phase proteins such CRP and SAA.[96] The functional effects of circulating IL-6, apart from increasing acute phase proteins, are not certain but there is evidence that they may be associated with skeletal muscle weakness. In an aging population with or without airway obstruction, plasma IL-6 concentrations are related to decreased muscle strength measured by quadriceps strength and exercise capacity.[97] In rats, infusion of IL-6 induces both cardiac failure and skeletal muscle weakness.[98] Increased circulating IL-6 concentrations are associated with several comorbid diseases, including ischemic heart disease, diabetes, and osteoporosis.

Plasma TNF-α and its soluble receptor (sTNFR75) are increased in patients with COPD,[99] and TNF-α is also released from circulating cells in patients with COPD with cachexia.[100] Circulating TNF-α seems to be related, at least in part, to hypoxaemia.[101] Increased systemic TNF-α has been implicated as a mechanism of cachexia and skeletal muscle weakness in patients with COPD. Chronic administration of TNF-α in animals results in cachexia, anemia, leukocytosis, and infiltration of neutrophils into organs such as heart, liver, and spleen.[102]

IL-1β has also been linked to cachexia, but increased plasma concentrations or decreased concentrations of its endogenous antagonist IL-1 receptor antagonist have not been found in COPD, although there is an association between COPD and a polymorphism of the IL-1β gene.[99]

CXCL8 and other CXC chemokines play an important role in neutrophil and monocyte recruitment in patients with COPD. Circulating CXCL8 concentrations are also increased in patients with COPD and have been related to muscle weakness.[103]

DEFECTIVE RESOLUTION OF INFLAMMATION AND REPAIR

The reason why inflammation persists in patients with COPD even after long-term smoking cessation is currently unknown, but if the molecular and cellular mechanisms for impaired resolution could be identified this may provide a novel approach

to COPD therapy. A major mechanism of airway obstruction in COPD is loss of elastic recoil caused by proteolytic destruction of lung parenchyma, so it is unlikely that this could be reversible by drug therapy. However, it might be possible to reduce the rate of progression by preventing the inflammatory and enzymatic disease process. In a similar way, the peribronchiolar fibrosis of small airways may not be reversible, but its progression may be halted by antifibrotic therapies.

Proresolving Lipid Mediators

Resolution of inflammation is an active process that may be facilitated by several endogenous proresolving mediators, including lipoxins, E-series resolvins, D-series protectins, and maresins, all of which are derived from polyunsaturated fatty acids and act on distinct receptors.[104] These mediators promote the resolution of neutrophilic inflammation by preventing neutrophil recruitment and enhancing neutrophil removal by efferocytosis. Endogenous mediators or stable structural analogues that activate the same receptors may have therapeutic potential in promoting resolution of inflammation in COPD. Maresin-1 is the most potent proresolving mediator that stimulates macrophage efferocytosis so a stable analogue of this mediator may be useful in COPD.[105]

Accelerated Aging

There is growing evidence that emphysema may be caused by accelerated aging of the lungs and it is hypothesized that accelerated aging may be caused by defective function of endogenous antiaging molecules, such as sirtuins and Forkhead box type O (FOXO) proteins, as a result of increased oxidative stress in the lung.[106] Sirtuin-1 (SIRT1) is markedly reduced in the peripheral lung of patients with COPD and in vitro this is mimicked by oxidative stress, which reduces activity and expression of SIRT1 resulting in increased expression of MMP9.[75] Resveratrol, a chemical found in red wine and derived from the skin of red fruits, is a weak sirtuin activator and prolongs the lifespan of several species, including mice. Resveratrol also suppresses the inflammatory response of macrophages from patients with COPD and epithelial cells, which is resistant to the effects of corticosteroids.[107,108] Resveratrol has poor bioavailability, which prompted a search for orally available and more potent sirtuin activators. An oral sirtuin activator, SRT2172, reverses the effects of cigarette smoking on MMP9 activity and inflammation in mice exposed to cigarette smoke.[75]

The pathway linking oxidative stress to reduced SIRT1 expression involved PI3K and activation of mammalian target of rapamycin (mTOR), which is inhibited by rapamycin and indirectly by metformin, both of which have been shown to have antiaging effects and to extend lifespan.[109] Through further understanding of the molecular pathways of cellular senescence in COPD it may be possible to identify novel targets and therapies to prevent disease progression and associated comorbidities.

Telomere shortening is associated with aging and there is evidence for reduced telomere length in lung and circulating cells from patients with COPD compared with normal smokers. This finding is associated with increased senescence and reduced telomerase activity in pulmonary endothelial cells of patients with COPD, which is linked to increased release of inflammatory mediators, such as IL-6, CXCL8, and CCL2.[110]

Airway Fibrosis

Increasing small airway fibrosis is an important mechanism of disease progression in COPD and is presumed to result from chronic inflammation, suggesting that effective antiinflammatory treatments should prevent fibrosis. Fibrosis may be mediated via the activation of fibroblasts by fibrogenic mediators, such as TGF-β, connective tissue growth factor (CTGF), and endothelin, which are secreted from epithelial cells and macrophages.[111] There are currently no effective antifibrotic therapies and no evidence that fibrosis can be reversed in COPD. Peroxisome proliferator-activated receptor gamma activators, such as rosiglitazone and pioglitazone, also have antifibrotic effects and may inhibit TGF-β signaling pathways in pulmonary fibroblasts.[112] Endothelin-1 is also profibrotic and several endothelin receptor antagonists have been found to be effective in inhibiting pulmonary fibrosis in animal models.[113]

IMPLICATIONS FOR FUTURE THERAPY

Inflammation is a driving mechanism for the progression of COPD, exacerbations, and probably associated comorbidities, including cardiovascular disease and lung cancer, which are the major causes of death among patients with COPD. However, this inflammatory process is largely resistant to the antiinflammatory effects of corticosteroids, although they are still widely used in the management of COPD, resulting in significant morbidity from side effects.

Reversal of Corticosteroid Resistance

This poor clinical response to corticosteroids in COPD reflects resistance to the antiinflammatory

effects of corticosteroids and may be explained by a reduction in HDAC2 as a result of oxidative and nitrative stress.[114] A reduction in HDAC2 activity and expression increases histone acetylation, with increased expression of inflammatory genes and reduction in the antiinflammatory effects of corticosteroids. The reduction in HDAC2 also results in increased acetylation of the glucocorticoid receptor, which prevents it from inhibiting NF-κB–driven inflammation.[27] A novel therapeutic strategy is reversal of this corticosteroid resistance through increasing the expression and activity of HDAC2 and this may be achieved with several existing and developing drugs.

Low concentrations of oral theophylline increase HDAC2 expression and activity in alveolar macrophages from patients with COPD and thus restore steroid responsiveness in these cells.[115] Mice exposed to cigarette smoke develop a steroid-resistant pulmonary inflammation that is suppressed by adding low-dose oral theophylline to the corticosteroid.[116] In patients with COPD, a low dose of oral theophylline combined with an inhaled corticosteroid was more effective in reducing inflammation in sputum than either drug alone.[117] This action of theophylline is independent of phosphodiesterase (PDE) inhibition and seems to be mediated by direct inhibition of oxidant stress–activated PI3Kδ.[116] Clinical trials with low-dose theophylline combined with corticosteroids are now underway. The tricyclic antidepressant nortriptyline also increases HDAC2 and reverses corticosteroid resistance by directly inhibiting PI3Kδ.[118] Oxidative stress is a major mechanism leading to corticosteroid resistance in COPD through reduced HDAC2. The antioxidant sulforaphane, increases HDAC2 and reverses corticosteroid resistance in mice exposed to cigarette smoke and in macrophages from patients with COPD.[72] Curcumin (diferuloylmethane), found in turmeric spice used in curry, also increases HDAC2 after it has been reduced by oxidative stress, and at concentrations lower than required for antioxidant effects.[119] Macrolides, including nonantibiotic macrolides, also reverse corticosteroid resistance through inhibiting PI3K signaling.[120]

New Antiinflammatory Therapies

There is an unmet need for safe and effective antiinflammatory treatments for COPD, but it has proved difficult to develop such drugs, despite the discovery of several logical targets.[2] Blocking individual cytokines with blocking antibodies or blocking chemokine receptors has so far proved to be disappointing in clinical studies. Broad-spectrum antiinflammatory treatments, such as PDE4 and proinflammatory kinase inhibitors, have often been poorly tolerated with side effects that limit the dose that can be used. For example, a PDE4 inhibitor, roflumilast, has significant antiinflammatory effects in COPD cells and animal models of COPD but the dose in patients with COPD is limited by side effects, so the therapeutic benefit is marginal.[121] It may be necessary to develop potent inhaled drugs in order to reduce systemic exposure and side effects, but it has proved difficult to discover inhibitors with high local potency that are retained within the lungs. If systemic inflammation is derived from peripheral lung inflammation, inhaled antiinflammatory treatments should reduce systemic inflammation and may therefore reduce or treat comorbidities.

New Pathways

There may be several coexisting mechanisms that interact in complex ways, so targeting a single pathway or mediator may not be effective in treating COPD, unless specific responder phenotypes are identified. It is important to identify the molecular mechanisms that underlie susceptibility so that only a minority of smokers develop COPD. So far, genome-wide association studies of COPD have been disappointing, but this may because the studies include all types of COPD. It is also likely that epigenetic mechanisms such as DNA methylation play an important role in disease susceptibility and these have yet to be explored.

As discussed earlier, there may be new disease processes, such as autoimmunity, defective phagocytosis, ineffective repair mechanisms, and cellular senescence, that may be targeted in the future, and there are several novel drugs in development.

The Need for Biomarkers

Clinical studies to show the effects of new drugs on disease progression are challenging because large numbers of patients studied over long periods (3 years) are necessary. It is important to identify biomarkers of disease activity or surrogate measurements that predict the clinical efficacy of antiinflammatory treatments in patients with COPD. Analysis of sputum parameters (cells, mediators, enzymes) or exhaled biomarkers (lipid mediators, ROS) may be useful as biomarkers of inflammation.[122]

Disease Phenotypes

It has long been recognized that COPD includes several different clinical and pathophysiologic phenotypes, but so far it has been different to

link any phenotype to a differential response to therapy. For example, roflumilast seems to work more effectively in a phenotype defined by disease severity, frequent exacerbations, and mucus hypersecretion, which may be a surrogate for neutrophilic inflammation.[123] Biomarkers that predict responsiveness to particular treatments are needed, particularly as treatments become more specific, such as targeting specific proteins. Patients with early disease seem to decline more rapidly may show better responses to therapy, whereas the focus of treatment currently is on patients with late disease who have the most symptoms. If effective and safe antiinflammatory therapies are developed for COPD it may be more useful to introduce these early in the course of the disease to prevent disease progression and possibly to reduce the burden of concomitant comorbidities. This approach is akin to the therapy for systemic hypertension and hypercholesterolemia in which treatment is given to prevent future risk.

Treating Acute Exacerbations

Because exacerbations represent a flare-up of the inflammation seen in stable disease, effective antiinflammatory treatments should also be able to reduce the intensity, duration, and consequences of an acute exacerbation.[124] This application of novel antiinflammatory therapies may be important, although it is challenging to test a novel antiinflammatory therapy in the management of an acute exacerbation. However, because such a treatment may be expected to be of only a few days' duration, this may make it possible to use therapies that would have side effects if used long-term.

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