

Inflammatory mechanisms in patients with chronic obstructive pulmonary disease



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Chronic obstructive pulmonary disease (COPD) is associated with chronic inflammation affecting predominantly the lung parenchyma and peripheral airways that results in largely irreversible and progressive airflow limitation. This inflammation is characterized by increased numbers of alveolar macrophages, neutrophils, T lymphocytes (predominantly T_H1, T_H1, and T_H17 cells), and innate lymphoid cells recruited from the circulation. These cells and structural cells, including epithelial and endothelial cells and fibroblasts, secrete a variety of proinflammatory mediators, including cytokines, chemokines, growth factors, and lipid mediators. Although most patients with COPD have a predominantly neutrophilic inflammation, some have an increase in eosinophil counts, which might be orchestrated by T_H2 cells and type 2 innate lymphoid cells through release of IL-33 from epithelial cells. These patients might be more responsive to corticosteroids and bronchodilators. Oxidative stress plays a key role in driving COPD-related inflammation, even in ex-smokers, and might result in activation of the proinflammatory transcription factor nuclear factor κ B (NF- κ B), impaired antiprotease defenses, DNA damage, cellular senescence, autoantibody generation, and corticosteroid resistance through inactivation of histone deacetylase 2. Systemic inflammation is also found in patients with COPD and can worsen comorbidities, such as cardiovascular diseases, diabetes, and osteoporosis. Accelerated aging in the lungs of patients with COPD can also generate inflammatory protein release from senescent cells in the lung. In the future, it will be important to recognize phenotypes of patients with optimal responses to more specific therapies, and development of biomarkers that identify the therapeutic phenotypes will be important. (*J Allergy Clin Immunol* 2016;138:16-27.)

Key words: Inflammation, macrophage, neutrophil, oxidative stress, cytokine, chemokine, autoantibody, nuclear factor κ B

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Chronic obstructive pulmonary disease (COPD) is a major global epidemic that is increasing throughout the world as populations age and survive previous causes of death.¹ COPD is now the fourth-ranked cause of death worldwide and predicted to become the fifth-ranked cause of disability, affecting approximately 10% of persons older than 45 years.² In developed countries COPD now affects female and male subjects equally, reflecting the equal prevalence of smoking.

It is now recognized that there are different clinical phenotypes of COPD, with some patients having predominantly small-airway disease and others having mainly emphysema. Other differences include age of onset, the frequency of exacerbations, and association with other diseases, such as chronic cardiovascular and metabolic diseases (comorbidities). Several attempts have been made to segregate patients with COPD into different clusters based on clinical and radiologic characteristics, but it has been difficult to identify these phenotypes in different populations, and there has been no link to underlying disease mechanisms (endotypes).^{3,4}

COPD is associated with chronic inflammation of the airways and lung parenchyma, which increases further during acute exacerbations and is also associated with systemic inflammation.⁵ Although the nature of this inflammation in lungs from patients with COPD has been well described, it is still uncertain how this relates to clinical outcomes, disease progression, and response to different therapies, and more research is needed to understand this better. Longitudinal studies of populations with COPD over many years have demonstrated that only about 50% of patients given a diagnosis of COPD have an accelerated decrease in lung function, whereas the remainder have a normal age-related decrease but start from a lower value, presumably because of impaired lung development.⁶ This implies that only half of patients with “COPD” have inflammation, whereas those with normal decreases in lung function presumably do not, although in most studies no distinction is made because the trajectory of lung function is unknown.

More research is urgently needed to understand different patterns of lung inflammation in patients with COPD and how these relate to clinical outcomes, response to therapy, and prognosis. In particular, it is necessary to understand how inflammation changes in response to different environmental stimuli and how it changes over time in the same patient. COPD of different causes can differ in terms of inflammation, but the COPD associated with *indoor air pollution* appears to have a very

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Terms in boldface and italics are defined in the glossary on page 17.

Abbreviations used

ASC: Apoptosis-associated speck-like protein containing a CARD
BAL: Bronchoalveolar lavage
COPD: Chronic obstructive pulmonary disease
EGFR: Epithelial growth factor receptor
HDAC: Histone deacetylase
ILC: Innate lymphoid cell
ILC2: Type 2 innate lymphoid cell
LT: Leukotriene
MMP: Matrix metalloproteinase
Nrf2: Nuclear erythroid 2-related factor 2
PG: Prostaglandin
ROS: Reactive oxygen species
SOD: Superoxide dismutase
VEGF: Vascular endothelial growth factor

similar pattern of inflammation to that seen in patients with smoking-related COPD, suggesting that the respiratory tract might respond to different risk factors in the same way.⁷ This review discusses the nature of inflammation in patients with COPD and the underlying molecular mechanisms that can identify new targets for more effective anti-inflammatory therapy in the future.⁸

PATHOLOGY OF COPD

The progressive airflow limitation in patients with COPD results from 2 major pathological processes: remodeling and narrowing of the small airways and destruction of the lung parenchyma with consequent loss of the alveolar attachments of these airways as a result of emphysema. These pathological

changes appear to be the consequence of chronic inflammation in the lung periphery, the intensity of which increases as the disease progresses.⁹ Even in patients with mild disease, there is obstruction and loss of the peripheral airways.¹⁰ Analysis of serial computed tomographic scans suggests that small-airway obstruction usually precedes the development of emphysema,¹¹ but the mechanisms for this are not clear. Small-airway obstruction and loss of alveolar attachments result in airway closure, and air trapping on expiration that is worsened by exercise and dynamic hyperinflation might account for exertional dyspnea, the major symptom of COPD, even in patients with mild disease.^{12,13} It is presumed that the peripheral location of inflammation in patients with COPD reflects the sites of deposition of inhaled irritant particles, such as cigarette and wood smoke. Indeed, in patients with COPD associated with household air pollution (*biomass smoke*) in developing countries, small-airway disease is predominant, whereas in cigarette smokers small airway disease and emphysema often coexist.¹⁴ The pattern of inhalation of irritants can also determine localization because wood smoke is usually inhaled tidally, whereas cigarette smoke is often inhaled deeply with a breath hold. This can be seen particularly in smokers of cannabis (marijuana) cigarettes, who can have marked emphysema.¹⁵

CHARACTERISTICS OF COPD-RELATED INFLAMMATION

In patients with COPD, there is a characteristic pattern of inflammation with increased numbers of neutrophils in the airway lumen and increased numbers of macrophages, T lymphocytes, and B lymphocytes.^{9,16,17} The inflammatory response in patients with COPD involves both innate and adaptive immune responses,

GLOSSARY

BIOMASS SMOKE: Indoor air pollution source resulting from burning of tobacco, wood, or other plant fuels. Burning of biomass results in polyaromatic hydrocarbon particulates that can be metabolized into oxidant species. Traditional biomass (wood, coal, charcoal, dung, and crop wastes) burned in open fires and traditional stoves are used by almost half the world's population and contribute to more than 1 million COPD-related deaths annually.

CYSTEINYL LEUKOTRIENES: Inflammatory mediators produced from arachidonic acid by 5-lipoxygenase in mast cells, eosinophils, and other inflammatory cells. Cysteinyl leukotrienes include leukotriene (LT) C₄, LTD₄, and LTE₄.

DEFENSINS: A distinct family of antimicrobial peptides produced by epithelial cells on mucosal surfaces, as well as by neutrophils, natural killer cells, and cytotoxic T lymphocytes. Defensins have direct antimicrobial activity, as well as the ability to activate inflammatory responses.

ELASTIN: A connective tissue protein similar to collagen that helps form a 3-dimensional basket-like structure around the alveoli and airways, allowing the lung to expand in all directions without developing excessive tissue recoil.

EXHALED BREATH CONDENSATES: A noninvasive technique for measuring airway inflammation obtained by cooling exhaled air. Exhaled breath condensate samples are thought to reflect contents of the airway lining fluid.

HISTONE: Water-soluble proteins rich in the basic amino acids lysine and arginine that complex with DNA in the nucleosomes of chromatin.

IL-10: A cytokine produced primarily by mononuclear phagocytic cells, as well as CD25⁺CD4⁺ regulatory T cells and T_H1 and T_H2 lymphocytes. IL-10 has many biologic functions aimed at maintaining homeostatic control of immune reactions, including inhibition of antigen-presenting cells, decreased MHC class II expression, decreased CD80 (T-cell costimulator) expression, inhibition of T_H1 and T_H2 cytokine production, and inhibition of IgE production and eosinophil activation.

INDOOR AIR POLLUTION: In addition to tobacco smoke, additional indoor pollutant sources include cooking and combustion, building materials, air conditioning, consumer products, heating, biologic agents, and particle resuspension. Specific products of indoor pollution include carbon monoxide, carbon dioxide, nitrogen dioxide, volatile organic compounds (eg, aldehydes), radon, lead, organic dusts, and microbial agents.

NLRP3 INFLAMMASOME: Also referred to as the NALP3 inflammasome, an enzyme complex that functions to activate the potent proinflammatory molecules IL-1, IL-18, and IL-33. Mutations in *NLRP3* result in the cryopyrin-associated periodic syndromes, such as Muckle-Wells syndrome, which involve inappropriate production of active IL-1 β .

$\gamma\delta$ T CELLS: Abundant in epithelial tissues, these T cells have a clonally distributed receptor composed of heterodimers of γ and δ chains. These receptors do not recognize MHC-associated peptide antigens and are not MHC restricted. $\gamma\delta$ T cells comprise only 5% of all T cells. The receptors for $\gamma\delta$ T cells have limited diversity.

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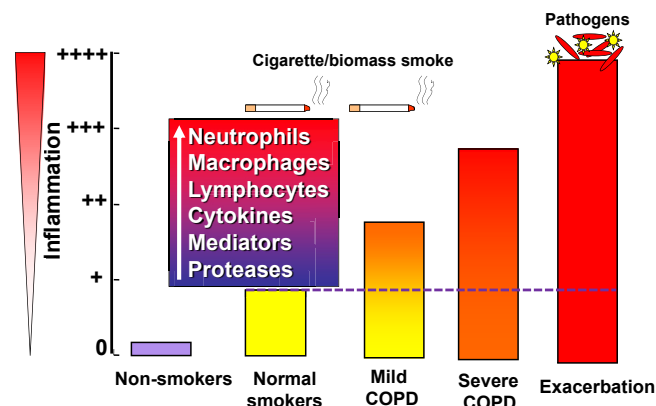


FIG 1. Amplification of inflammation in patients with COPD compared with that seen in smokers who do not have airflow obstruction. Once established, this inflammation persists, even after smoking cessation. Inflammation is further increased in acute exacerbations triggered by bacteria or viruses.

which are linked through activation of dendritic cells.¹⁸ A similar pattern of inflammation and mediator expression is found in smokers without airflow limitation, but in patients with COPD, this inflammation is amplified and even further increased during acute exacerbations or precipitated by bacterial or viral infection (Fig 1). The molecular basis for the amplification of inflammation is not yet fully understood but might be, at least in part, determined by genetic and epigenetic factors. The molecular mechanisms of amplification might determine which smokers are susceptible to the development of airway obstruction. Cigarette smoke and other irritants inhaled into the respiratory tract might activate surface macrophages and airway epithelial cells to release multiple chemotactic mediators, particularly chemokines, which attract circulating neutrophils, monocytes, and lymphocytes into the lungs. This inflammation persists even when smoking is stopped, suggesting that there are self-perpetuating mechanisms, although these have not yet been elucidated.¹⁹ It is possible that long-lived memory T cells, bacterial colonization, or autoimmunity might drive the persistent inflammation seen in patients with COPD.

INFLAMMATORY CELLS

The inflammation seen in the lungs of patients with COPD involves both innate immunity (neutrophils, macrophages, eosinophils, mast cells, natural killer cells, $\gamma\delta$ T cells, innate lymphoid cells, and dendritic cells) and adaptive immunity (T and B lymphocytes), but also, there is 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 TNF- α , IL-1 β , IL-6, GM-CSF, and CXCL8 (IL-8).²⁰ Epithelial cells in the small airways express TGF- β , which then induces local fibrosis. Vascular endothelial growth factor (VEGF) appears to be necessary to maintain alveolar cell integrity, and blockade of VEGF receptors in rats induces apoptosis of alveolar cells and an emphysema-like pathology.¹⁴ A reduction in peripheral lung VEGF concentrations is found in

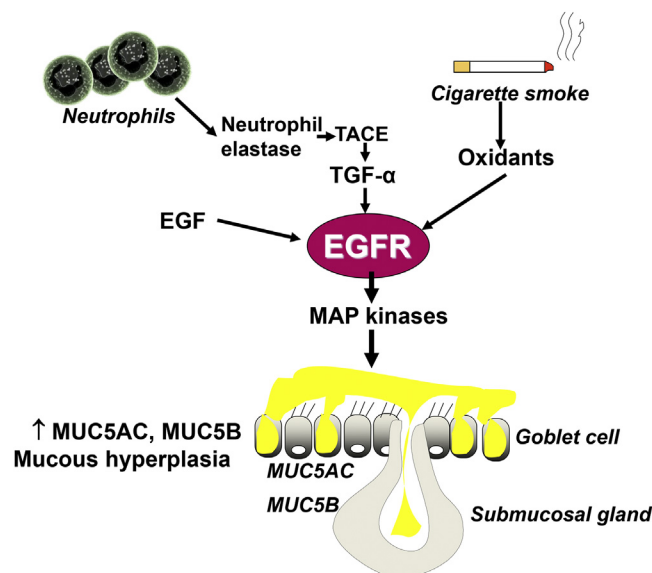


FIG 2. Mucus hypersecretion and hyperplasia in patients with COPD. EGFRs can be activated by neutrophils through the effect of neutrophil elastase on TNF- α -converting enzyme (TACE), which releases TGF- α , a ligand for EGFRs. EGFRs can also be activated indirectly through oxidative stress. EGFRs activate mitogen-activated protein (MAP) kinases, which increase expression of the mucin genes MUC5AC and MUC5B and also lead to hyperplasia of goblet cells and submucosal glands.

smokers and patients with COPD, but levels of another growth factor, hepatocyte growth factor, are increased in smokers and therefore might protect against the effect of reduced VEGF levels on alveolar integrity. However, in patients with COPD, both VEGF and hepatocyte growth factor levels are reduced, which might contribute to development of emphysema.²¹ Airway epithelial cells are also important in defense of the airways, with mucus production from goblet cells and secretion of antioxidants, antiproteases, and *defensins*/cathelicidins. Cigarette smoke and other noxious agents might impair these responses of the airway epithelium, increasing susceptibility to infection. The airway epithelium in patients with chronic bronchitis and COPD often shows squamous metaplasia, which can result from increased proliferation of basal airway epithelial cells, but the nature of the growth factors involved in epithelial cell proliferation, the cell cycle, and differentiation in patients with COPD are not yet certain. Epithelial growth factor receptors (EGFRs) show increased expression in airway epithelial cells of patients with COPD and might contribute to basal cell proliferation, resulting in squamous metaplasia and an increased risk of bronchial carcinoma.²² Club (Clara) cells can act as progenitor cells in the peripheral airways and might be susceptible to damage from inhaled irritants. Conditional depletion of club cells in mice leads to squamous cell metaplasia and peribronchiolar fibrosis, similar to the pathology of COPD.²³ Club cell secretory protein is deficient in patients with COPD.²⁴

Mucus hyperplasia is a feature of many patients with COPD and is a response to chronic airway irritation by cigarette smoke and other pollutants. EGFRs play an important role on mucus hyperplasia and secretion and can be activated by neutrophilic inflammation through neutrophil elastase secretion, which releases TGF- α (Fig 2).²⁵ Oxidant stress is also able to activate EGFRs and induce mucus hypersecretion.²⁶ An EGFR inhibitor is effective in blocking LPS- and TNF- α -induced expression of MUC5AC in human airway epithelial cells and inhibiting

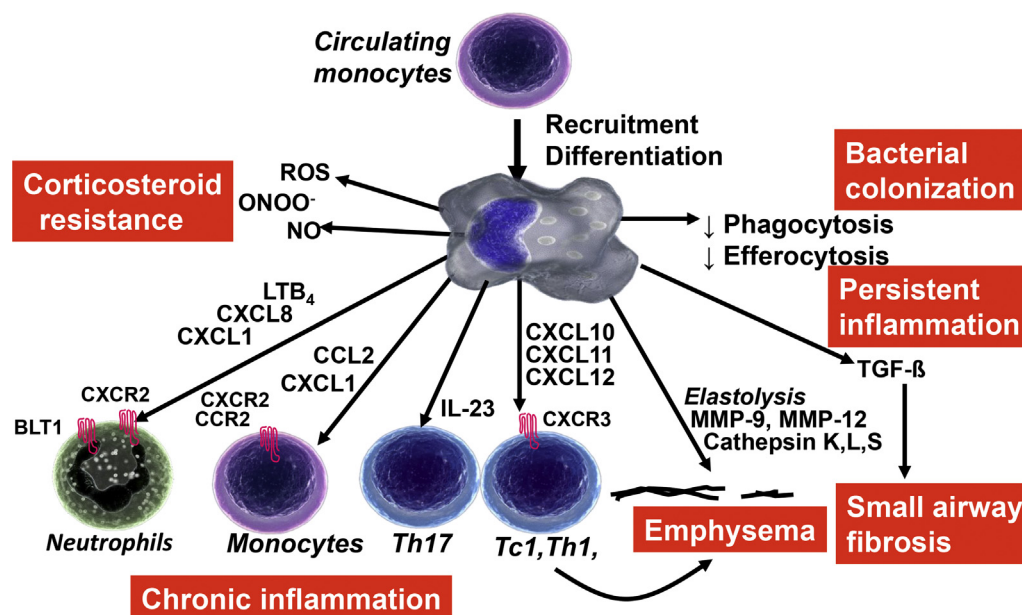


FIG 3. Central role of alveolar macrophages in patients with COPD. Alveolar macrophages are derived from circulating monocytes, which differentiate within the lung. They secrete many inflammatory proteins that can orchestrate the inflammatory process in patients with COPD. Neutrophils can be attracted by CXCL8, CXCL1, and LTB₄; monocytes by CCL2; and T_H1 and T_H17 lymphocytes by CXCL10, CXCL11, and CXCL12. Release of elastolytic enzymes, including MMPs and cathepsins, cause elastolysis and contribute to emphysema together with cytotoxic T cells. Release of TGF-β1 can induce fibrosis of the small airways. Macrophages generate ROS and nitric oxide (NO), which together form peroxynitrite (ONOO⁻) and might contribute to corticosteroid resistance. Defective bacterial phagocytosis can lead to bacterial colonization and defective efferocytosis.

LPS-induced mucus secretion and hyperplasia in rats exposed to cigarette smoke.²⁷ However, an EGFR inhibitor (BIBW2948) was ineffective in reducing mucin gene expression in patients with COPD and was tolerated poorly.²⁸

Macrophages

Macrophages play a key role in orchestrating chronic inflammation in patients with COPD (Fig 3).²⁹ Macrophage numbers are markedly increased (5- to 10-fold) in the airways, lung parenchyma, bronchoalveolar lavage (BAL) fluid, and sputum of 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 the severity of emphysema. Macrophages can be activated by cigarette smoke extract to release inflammatory mediators, including TNF-α, CXCL1, CXCL8, CCL2 (monocyte chemoattractant protein 1), leukotriene (LT) B₄, and reactive oxygen species (ROS). Alveolar macrophages also secrete elastolytic enzymes, including matrix metalloproteinases (MMPs) 2, 9, and 12; cathepsins K, L, and S; and neutrophil elastase taken up from neutrophils.³⁰ 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. 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 healthy control subjects.

There are different phenotypes of macrophages that can be differentially activated and with different responses. The murine

M1 or “classically activated” macrophages are proinflammatory, whereas M2 or “alternatively activated” macrophages are more anti-inflammatory, release IL-10, and show marked phagocytic activity.³¹ However, these distinctions are less clear in human macrophages, and the surface markers of these phenotypes are less distinct. In general, it is likely that M1-like macrophages predominate in patients with COPD, but further studies are needed.³²

MMP-9 is the predominant elastolytic enzyme secreted by alveolar macrophages from patients with COPD. Most of the inflammatory proteins upregulated in macrophages from patients with COPD are regulated by the transcription factor NF-κB, which is activated in alveolar macrophages of patients with COPD, particularly during exacerbations.³³ The increased numbers of macrophages in the lungs of smokers and patients with COPD is due to increased recruitment of monocytes from the circulation in response to the monocyte-selective chemokines CCL2 and CXCL1, levels of which are increased in the sputum and BAL fluid of patients with COPD.³⁴

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, and appears to be due to greater receptor turnover.³⁵ Macrophages also release CXCL9, CXCL10 and CXCL11, which are chemotactic for CD8⁺ T_H1 and CD4⁺ T_H1 cells through interaction with the chemokine receptor CXCR3 expressed on these cells.³⁶ Macrophages from patients with COPD release more inflammatory proteins than macrophages from normal smokers and nonsmokers, indicating increased activation.³⁷

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

COPD.³⁸ *In vitro* release of CXCL8, TNF- α , and MMP-9 from macrophages from healthy subjects and normal smokers is inhibited by corticosteroids, whereas they are relatively ineffective in macrophages from patients with COPD.³⁷ The reasons for resistance to corticosteroids in patients with COPD might be the marked reduction in activity of histone deacetylase (HDAC) 2, which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes.³⁹ The reduction in HDAC activity in macrophages is correlated with increased secretion of cytokines, such as TNF- α and CXCL8, and reduced corticosteroid response.

Both alveolar and monocyte-derived macrophages from patients with COPD show reduced phagocytic uptake of bacteria, and this might be a factor in determining chronic colonization of the lower airways by bacteria, such as *Haemophilus influenzae* or *Streptococcus pneumoniae*.⁴⁰ Macrophages from patients with COPD are also defective in taking up apoptotic cells, and this might contribute to the failure to resolve inflammation in patients with COPD.⁴¹ The nature of this defect in phagocytosis is not fully understood but appears to be due to a defect in microtubular function, which is required for phagocytosis, rather than any abnormality of recognition of the phagocytosed particles.⁴² Bacterial colonization of the lower airways, which is found in at least 50% of patients with COPD, can predispose to increased acute exacerbations and also to increased risk of community-acquired pneumonia in patients with COPD.⁴³

Neutrophils

Increased numbers of activated neutrophils are found in the sputum and BAL fluid of patients with COPD and correlate with disease severity, although few neutrophils are seen in the airway wall and lung parenchyma, likely reflecting their rapid transit through these tissues. Smoking has a direct stimulatory effect on granulocyte production and release from bone marrow and survival in the respiratory tract, possibly mediated by the hematopoietic growth factors GM-CSF and granulocyte colony-stimulating factor released from lung macrophages. Neutrophil recruitment to the airways and parenchyma involves initial adhesion to endothelial cells through E-selectin, which is upregulated on endothelial cells in the airways of patients with COPD.⁴⁴ Adherent neutrophils migrate into the respiratory tract under the direction of various neutrophil chemotactic factors, including LTB₄, CXCL1, CXCL5, and CXCL8, levels of which are increased in airways of patients with COPD. These chemotactic mediators can be derived from alveolar macrophages, T cells, and epithelial cells, but the neutrophil itself might 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 and human neutrophil lipocalin, in the sputum supernatant.⁴⁵ Neutrophils secrete serine proteases, including neutrophil elastase, cathepsin G, and proteinase-3, as well as MMP-8 and MMP-9, which might 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.⁴⁶ There is a marked increase in neutrophil numbers in the airways of patients with acute COPD exacerbations, accounting for the increased purulence of sputum. This might reflect increased production of neutrophil chemotactic factors, including LTB₄ and

CXCL8.⁴⁷ Neutrophils from patients with COPD show marked abnormalities in chemotactic response, with increased migration but reduced accuracy, which is reminiscent of the abnormal monocyte chemotactic responses.⁴⁸

Eosinophils

Although eosinophils are the predominant leukocyte in asthmatic patients, their role in patients with COPD is much less certain. Increased eosinophil numbers have been described in the airways and BAL fluid of patients with stable COPD, whereas others have not found increased numbers in airway biopsy specimens, BAL fluid, or induced sputum.³⁹ The presence of eosinophils in patients with COPD predicts a more favorable therapeutic response to bronchodilators and corticosteroids and might indicate coexisting asthma or asthma-COPD overlap.⁴⁹⁻⁵¹ Approximately 15% of patients with COPD appear to have clinical features of asthma.⁵² The mechanisms for increased eosinophil counts in patients with COPD are uncertain, but there has recently been considerable interest in the role of type 2 innate lymphoid cells (ILC2s), which might be important in intrinsic asthma but also in COPD.^{53,54} ILC2s can be regulated by epithelial mediators, such as IL-33, released as a result of epithelial cell injury (Fig 4). IL-33 expression is increased in basal epithelial progenitor cells in patients with COPD and is associated with increased expression of IL-13 and the mucin gene 5AC.⁵⁵ There appears to be considerable plasticity in innate lymphoid cells (ILCs) and evidence that ILC2s can convert to type 1 ILCs, which release IFN- γ after viral infection and exposure to IL-12 and IL-18.⁵⁶

The presence of eosinophils in the airways might indicate a better response to corticosteroid therapy and therefore might define a therapeutic phenotype of patients with COPD. Sputum concentrations of IL-5 are correlated with sputum eosinophil numbers, and both are reduced by oral corticosteroids.⁵⁷ There is a relationship between bronchial wall and blood eosinophil numbers, and therefore this might make it feasible to use blood eosinophil counts as a biomarker for steroid responsiveness in patients with COPD.⁵⁸ In retrospective analyses of clinical trials, patients with increased blood eosinophil numbers (>2% or >200/ μ L) show a greater reduction in exacerbations than patients with lower eosinophil numbers, although there was no difference in lung function or symptoms.^{59,60} Increased eosinophil numbers have been reported in bronchial biopsy specimens and BAL fluid during acute exacerbations of chronic bronchitis.⁶¹

Lymphocytes

There is an increase in total numbers of T lymphocytes in the lung parenchyma and peripheral and central airways of patients with COPD, with the greater increase in CD8⁺ than CD4⁺ cells.^{9,36} There is a correlation between T-cell numbers and the amount of alveolar destruction and 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 numbers of T cells, mainly CD8⁺ (T_C1) cells, in patients with COPD.⁶² There is also an increase in the absolute number of CD4⁺ (T_H1) T cells, although in smaller numbers, in the airways of smokers with COPD, and these cells express activated signal transducer and activator of transcription

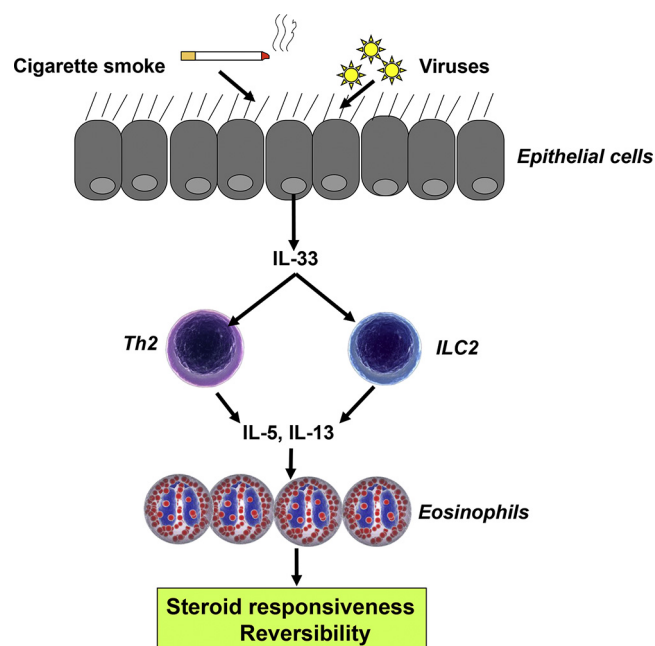


FIG 4. Eosinophilic inflammation in patients with COPD. Both T_H2 cells and ILCs can cause eosinophilic inflammation through IL-5 and IL-13 release and might result in a better response to corticosteroids and bronchodilators. T_H2 cells and ILC2s can be activated by IL-33 from damaged epithelial cells.

4, a transcription factor essential for activation and commitment of the T_H1 lineage.⁶³ Numbers of $CD4^+$ T_H17 cells, which secrete IL-17A and IL-22, are also increased in the airways of patients with COPD and might play a role in orchestrating neutrophilic inflammation.^{64,65} T_H17 cells can 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, levels of which are increased in patients with COPD.⁶⁶ 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 5).⁶⁷ $CD8^+$ cell numbers are typically increased in patients with airway infections, and it is possible that chronic bacterial colonization of the lower respiratory tract of patients with COPD is responsible for this inflammatory response.

As discussed above, ILCs are now thought to play an important role in regulation of lung immunity and might be regulated through danger signals and cell damage.⁶⁸ There is considerable plasticity between different subtypes of ILCs in response to different triggers and cytokines. All 3 types of ILCs have been identified in human lungs.⁵⁴ In patients with COPD, there is an increase in numbers of ILC3s, which secrete IL-17 and IL-22, and these cells might play a role in driving neutrophilic inflammation.

Autoimmune mechanisms can also be involved. Cigarette-induced lung injury could uncover previously sequestered autoantigens, or cigarette smoke itself might damage lung interstitial and structural cells and make them antigenic. Oxidative stress can result in formation of carbonylated proteins, which are antigenic, and several anticarbonylated protein antibodies have been found in the circulation of patients with

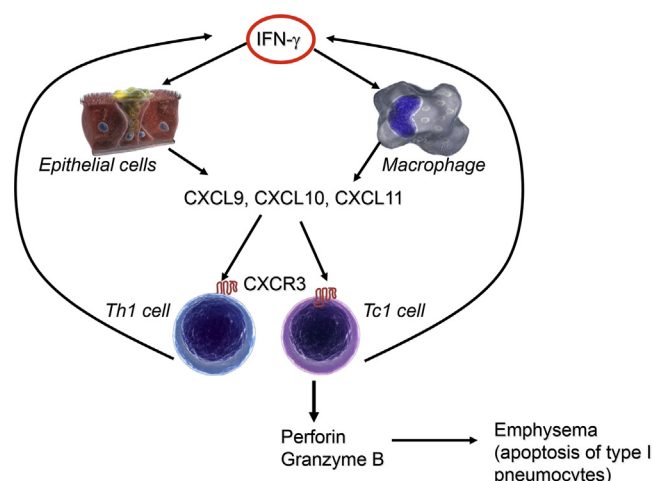


FIG 5. Lymphocytes in patients with COPD. Epithelial cells and macrophages release the chemokines CXCL9, CXCL10, and CXCL12, which activate CXCR3 on T_H1 and T_C1 cells, which releases IFN- γ , which in turn causes further release of these chemokines. T_C1 cells are cytotoxic because of the release of perforin and granzyme B, which can contribute to alveolar cell apoptosis and the development of emphysema.

COPD, particularly in those with severe disease.⁶⁹ Anti-endothelial antibodies have also been detected.⁷⁰ Autoantibodies can cause cell damage through the binding of complement, levels of which are increased in the lungs of patients with COPD.⁶⁹ Citrullinated proteins are also described in the lungs of patients with COPD and might induce autoantibody formation, as in patients with rheumatoid arthritis.⁷¹

$CD8^+$ 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 patients with emphysema.⁷² There is evidence for immunologic senescence in patients with COPD, with increased numbers of T cells with no expression of the costimulatory receptor CD28 ($CD4/CD28^{\text{null}}$ and $CD8/CD28^{\text{null}}$ cells), and these cells release increased amounts of perforins and granzyme B.^{73,74} Senescence of T cells in patients with COPD is associated with reduced expression of HDAC2.⁷⁵

Numbers of B lymphocytes are also increased in the lungs of patients with COPD, particularly in those with severe disease. B cells are organized into lymphoid follicles, which are located in peripheral airways and lung parenchyma.⁹ B-cell activating factor is an important regulator of B-cell function and hyperplasia, and its expression is increased in lymphoid follicles of patients with COPD.⁷⁶

Dendritic cells

Dendritic cells are an important link between innate and adaptive immunity. The airways and lungs contain a rich network of dendritic cells localized near the surface, so that they are ideally located to signal the entry of inhaled foreign substances. Dendritic cells can activate a variety of other inflammatory and immune cells, including macrophages, neutrophils, and T and B lymphocytes, and therefore dendritic cells might play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents. Dendritic cells appear to be activated in the lungs of patients with COPD⁷⁷ and are linked to disease severity.⁷⁸ Numbers of dendritic cells are increased in the lungs

of patients with COPD, and cigarette smoke increases their survival *in vitro*.⁷⁹

INFLAMMATORY MEDIATORS

Many inflammatory mediators have been implicated in COPD, including lipids, free radicals, cytokines, chemokines, and growth factors.⁸⁰ 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 a significant clinical effect. Similar mediators that are found in the lungs of patients with COPD might also be increased in the circulation, and this systemic inflammation could underlie and potentiate comorbidities.

Lipid mediators

The profile of lipid mediators in *exhaled breath condensates* of patients with COPD shows an increase in prostaglandin (PG) and LT levels. There is a significant increase in PGE₂ and PGF_{2α} concentrations and an increase in LTB₄ concentrations but not *cysteinyl leukotrienes*.⁸¹ This is a different pattern than that seen in asthmatic patients, which has increases in thromboxane and cysteinyl leukotriene levels. LTB₄ concentrations are increased in induced sputum and further increased in sputum and exhaled breath condensate during acute exacerbations.^{47,82} LTB₄ is a potent chemoattractant of neutrophils, acting through high-affinity BLT₁ receptors. A BLT₁ receptor antagonist reduces the neutrophil chemotactic activity of sputum by approximately 25%.⁸² BLT₁ receptors have also been identified on T lymphocytes, and there is evidence that LTB₄ is also involved in recruitment of T cells.

Cytokines

Cytokines are mediators of chronic inflammation, and several have been implicated in patients with COPD.⁸³ There is an increase in concentrations of TNF-α in induced sputum in patients with stable COPD, with a further increase during exacerbations. 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 found in some patients with severe disease. TNF-α is a potent activator of NF-κB, and this might amplify the inflammatory response. Unfortunately, anti-TNF therapies have not been proved effective in patients with COPD and might have serious adverse effects.⁸⁴ The reason for the failure of anti-TNF therapies in patients with COPD is presumably because other proinflammatory cytokines drive the inflammatory process.

Inflammasome

Inflammasomes are multiprotein signaling complexes that regulate the expression of the proinflammatory cytokines IL-1β and IL-18 in response to external and endogenous danger signals, resulting in neutrophilic inflammation.⁸⁵ Most attention has focused on *NLRP3 inflammasomes*, which are activated in patients with several inflammatory lung diseases (Fig 6).⁸⁶ The adapter protein apoptosis-associated speck-like protein containing a CARD (ASC) is an important component of the NLRP3 inflammasome, which recruits pro-caspase-1 to the

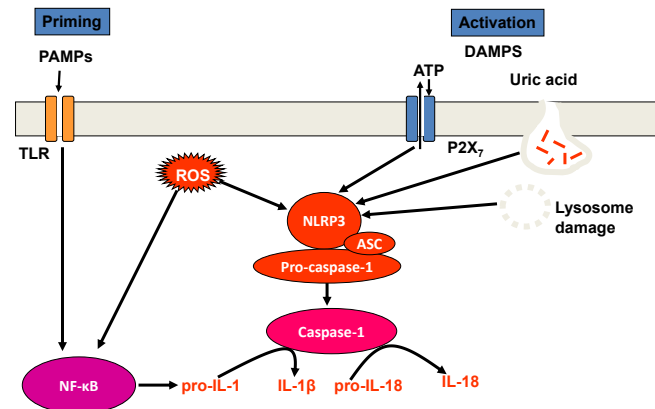


FIG 6. Inflammasome activation in patients with COPD. The NLRP3 inflammasome is activated by 2 signals. The priming signal is activated by pathogens through pathogen-activated molecular patterns (PAMPs) with the generation of pro-IL-1β and pro-IL-18 by means of activation of NF-κB. The activation signal can include ATP (through P2X₇-receptors) and other damage-activated molecular patterns (DAMPs), such as uric acid. This causes recruitment of the adapter protein ASC and pro-caspase-1 to generate caspase-1, which releases active IL-1β and IL-18.

protein complex and is increased in lungs of patients with COPD.⁸⁷ ASC accumulation is associated with the formation of extracellular “specks,” which perpetuate the generation of IL-1β outside the cell. However, in patients with stable COPD, no increases in the NLRP3 inflammasome were found, apart from a small increase in patients with severe disease, possibly because of an increase in levels of the inflammasome inhibitory molecules NALP7 and IL-37. It is more likely that NLRP3 inflammasome activation is linked to acute exacerbations, in which pathogens, oxidative stress, and ATP, all of which activate the NLRP3 inflammasome, are increased. The minimal role of the inflammasome in patients with stable COPD is underlined by the failure of the IL-1β blocking antibody canakinumab to provide any clinical benefit in patients with stable COPD.⁸⁸

Levels of IL-17 and other T_H17 cytokines are also increased in sputum and airways of patients with COPD and might play a role in orchestrating neutrophilic inflammation in the lungs.^{64,89} These cytokines can also be released from ILC3s, numbers of which are increased in patients with COPD.⁵⁴

Chemokines

Several chemokines have been implicated in patients with COPD and have been of particular interest because chemokine receptors are G protein-coupled receptors for which small-molecule receptor antagonists have been developed.⁹⁰ 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 and is chemotactic for neutrophils through the high-affinity 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 might be more important as a chemoattractant than CXCL8, acting through CXCR2, which is expressed on neutrophils and monocytes.³⁴ CXCL1 induces significantly more chemotaxis of monocytes of patients with COPD compared with those of normal smokers, and this might reflect increased

turnover and recovery of CXCR2 in monocytes of patients with COPD.³⁵ 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 expression is increased in concentration in sputum and BAL fluid from patients with COPD and plays a role in monocyte chemotaxis through activation of CCR2.³⁴ The chemokine CCL5 (RANTES) is also expressed in the airways of patients with COPD during exacerbations and activates CCR5 on T cells and CCR3 on eosinophils, which might account for the increased eosinophil and T-cell numbers seen in the walls of large airways that have been reported during exacerbations of chronic bronchitis. As discussed above, CXCR3 expression is upregulated on T_C1 and T_H1 cells of patients with COPD, with increased expression of the ligands CXCL9, CXCL10, and CXCL11.⁶⁶ CXCR3 ligands induce increased chemotaxis of monocytes and lymphocytes from patients with COPD, which might reflect increased CXCR3 expression in patients with COPD.⁹¹

Proteases

The increase in elastase activity in patients with COPD might contribute to the development of emphysema and neutrophilic inflammation through generation of chemotactic peptides, such as acetylated Pro-Gly-Pro (matrikines), which are potent neutrophil chemoattractants that activate CXCR2.⁹² This might be self-perpetuating because neutrophils release MMP-9, which in turn generates more matrikine.⁹³ Human neutrophil elastase not only has elastolytic activity but is also a potent stimulant of mucus secretion in the airways, as mediated through EGFRs.⁹⁴ MMP-9 is the predominant elastolytic enzyme in patients with COPD and is secreted from macrophages, neutrophils, and epithelial cells.

OXIDATIVE STRESS AS A MAJOR DRIVING MECHANISM

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 patients with COPD.⁹⁵ Inflammatory and structural cells, including neutrophils, macrophages, and epithelial cells, which are activated in the airways of patients with COPD, produce ROS. Superoxide anions ($O_2^{\cdot-}$) are generated by NADPH oxidase and converted to hydrogen peroxide (H_2O_2) by superoxide dismutases (SODs). H_2O_2 is then converted to water by catalase. $O_2^{\cdot-}$ and H_2O_2 might interact in the presence of free iron to form the highly reactive hydroxyl radical ($\cdot OH$). $O_2^{\cdot-}$ can also combine with NO to form peroxynitrite, which also generates $\cdot OH$. Oxidative stress leads to oxidation of arachidonic acid and formation of a new series of prostanoid mediators, called isoprostanes, which can exert significant functional effects, including bronchoconstriction and plasma exudation.⁹⁶ Peroxynitrite levels are also increased in the breath of patients with COPD.⁹⁷ Nitric oxide levels can be increased in the peripheral lungs of patients with COPD and appear to be linked to increased expression of inducible and neural nitric oxide synthases (NOS2 and NOS1).⁹⁸

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract, including

catalase, SOD, and glutathione, formed by the enzyme γ -glutamyl cysteine ligase and glutathione synthetase. In the lung intracellular antioxidants are expressed at relatively 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 (α -tocopherol), uric acid, lactoferrin, and extracellular SOD3, which is highly expressed in the 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 lungs and cells from patients with COPD, Nrf2 is not appropriately activated, despite high levels of oxidative stress in the lungs,⁹⁹ and this can be related to increased acetylation caused by decreased HDAC2 levels.¹⁰⁰

ROS have wide-ranging effects on the airways and parenchyma and increase the inflammatory response. ROS activate NF- κ B, 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.¹⁰¹ Oxidative stress can also impair the function of antiproteases, such as α_1 -antitrypsin and secretory leukoprotease inhibitor, and thereby accelerates the breakdown of *elastin* in lung parenchyma. Oxidative stress markedly reduces HDAC2 activity and expression through activation of phosphoinositide 3-kinase δ and peroxynitrite-induced nitration of tyrosine residues.³⁹ This 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 lungs and cells of patients with COPD might underlie the accelerated aging response seen in patients with COPD.^{102,103}

Oxidative stress can also predispose to lung cancer through activation of growth factors and DNA damage.¹⁰⁴ As discussed above, oxidative stress leads to formation of carbonylated proteins, which might be antigenic and stimulate the development of autoantibodies in patients with COPD, which can cause persistence of inflammation.⁶⁹

SYSTEMIC INFLAMMATION IN PATIENTS WITH COPD

Patients with COPD, particularly when the disease is severe and during exacerbations, have evidence of systemic inflammation, which is measured either as increased circulating cytokine, chemokine, and acute-phase protein levels or as abnormalities in circulating cells.^{105,106} Persistent inflammation is associated with poorer clinical outcomes. Smoking itself can cause systemic inflammation (eg, 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 "spillover" from inflammation in the peripheral lung or are a parallel abnormality or related to some comorbid disease that then has effects on the lung. In any case the systemic inflammation seen in patients with COPD might contribute to its systemic manifestations and could worsen comorbid diseases.

In a large population study systemic inflammation (increased C-reactive protein, fibrinogen, and leukocyte levels) was associated with a 2- to 4-fold increased risk of cardiovascular disease, diabetes, lung cancer, and pneumonia, although not with depression.¹⁰⁷ By measuring 6 inflammatory markers (C-reactive protein, IL-6, CXCL8, fibrinogen, TNF- α , and leukocytes), 70% of patients with COPD had some components of systemic inflammation, and 16% had persistent inflammation.¹⁰⁶ Patients with persistent systemic inflammation had increased mortality and more frequent exacerbations. Systemic inflammation appears to relate to accelerated decrease in lung function and is increased further during exacerbations.¹⁰⁸

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 might provide a novel approach to COPD therapy. A major mechanism of airway obstruction in patients with COPD is loss of elastic recoil because of proteolytic destruction of lung parenchyma, and therefore 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. Similarly, peribronchiolar fibrosis of the small airways might not be reversible, but its progression can be halted by antifibrotic and anti-inflammatory therapies.

Proresolving lipid mediators

Resolution of inflammation is an active process that can 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.¹⁰⁹ These mediators promote the resolution of neutrophilic inflammation by preventing neutrophil recruitment and enhancing neutrophil removal by means of efferocytosis. Endogenous mediators or stable structural analogs that activate the same receptors can have therapeutic potential in promoting resolution of inflammation in patients with COPD. Maresin-1 is the most potent proresolving mediator that stimulates macrophage efferocytosis, and therefore a stable analogue of this mediator might be useful in patients with COPD.¹¹⁰ Defective efferocytosis by macrophages in patients with COPD might prevent resolution of inflammation and could be the same defect that reduces bacterial phagocytosis.^{41,42}

Accelerated aging

There is growing evidence that emphysema might be due to accelerated aging of the lungs, and it is hypothesized that accelerated aging might be due to defective function of endogenous antiaging molecules, such as sirtuins and FOXO proteins, as a result of increased oxidative stress in the lung.^{103,111} Sirtuin-1 expression is markedly reduced in the peripheral lungs of patients with COPD, and *in vitro* this is mimicked by oxidative stress, which reduces the activity and expression of sirtuin-1, resulting in increased expression of MMP-9.¹⁰² The pathway linking oxidative stress to reduced sirtuin-1 expression involved

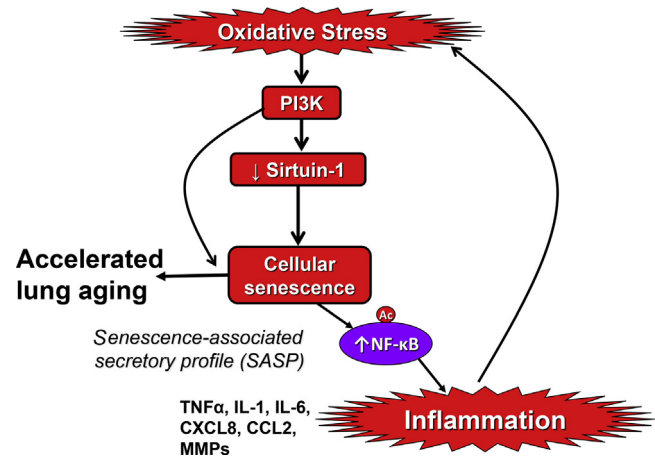


FIG 7. Accelerated aging and inflammation in patients with COPD. Oxidative stress drives accelerated aging through activation of phosphoinositide 3-kinase (PI3K) and reduction in sirtuin-1 levels, which leads to cellular senescence and release of inflammatory proteins, which further increase oxidative stress.

phosphoinositide 3-kinase and activation of mammalian target of rapamycin, which is activated in patients with COPD and inhibited by rapamycin.¹¹² Because the same molecular pathways are shared by many of the comorbidities of COPD, which are also characterized by accelerated aging, it might be possible to identify novel targets and therapies to prevent disease progression and associated comorbidities.¹¹¹ Cellular senescence is found in the lungs of patients with COPD,¹¹³ and senescent cells are known to secrete inflammatory proteins, including TNF- α , IL-1, IL-6, CXCL8, CCL2 and MMPs. This senescence-associated secretory profile might account for the chronic low-grade inflammation found in patients with COPD (Fig 7).¹¹⁴

Airway fibrosis

Increasing small-airway fibrosis is an important mechanism of disease progression in patients with COPD and is presumed to result from chronic inflammation, suggesting that effective anti-inflammatory treatments should prevent fibrosis. Fibrosis can be mediated through activation of fibroblasts by fibrogenic mediators, such as TGF- β , connective tissue growth factor, and endothelin, which are secreted from epithelial cells and macrophages.¹¹⁵ Endothelin-1 is also profibrotic, and several endothelin receptor antagonists inhibit pulmonary fibrosis in animal models.

FUTURE IMPLICATIONS

There is a need to identify phenotypes of COPD that respond to specific therapies, and this will involve recognizing disease endotypes and biomarkers that predict response.

As discussed above, some patients with COPD have eosinophilic inflammation, and this can be recognized by increased blood eosinophil numbers, which might indicate patients who have a better therapeutic response to inhaled corticosteroids. Prospective studies are needed to define the cutoff point for blood eosinophils that indicates a good steroid response. Furthermore, those patients with COPD without increased eosinophil numbers should probably have inhaled corticosteroids withdrawn, which appears to be safe, even in high-risk patients treated with high doses of inhaled corticosteroids.¹¹⁶

Although inflammation is believed to be a key driving mechanism for COPD progression and exacerbations, it is also apparent in elderly patients with COPD that cellular senescence can lead to low-grade inflammation, which might then drive further disease progression.¹¹⁴

It is now recognized that there might be several coexisting mechanisms in COPD that interact in complex ways so that targeting a single pathway or mediator might 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 will have COPD. Thus far, genome-wide association studies of COPD have been disappointing, but this might be 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 have yet to be explored.

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 will predict the clinical efficacy of anti-inflammatory treatments in patients with COPD. Analysis of sputum parameters (cells, mediators, and enzymes) or exhaled biomarkers (lipid mediators and ROS) might be useful as biomarkers of inflammation.¹¹⁷

COPD includes several different clinical and pathophysiologic phenotypes, but thus far, it has been difficult to link any phenotype to a differential response to therapy, apart from the patients with eosinophilia discussed above. The phosphodiesterase 4 inhibitor roflumilast appears to work more effectively in a phenotype defined by disease severity, frequent exacerbations, and mucus hypersecretion, which might be a surrogate for neutrophilic inflammation.¹¹⁸ Biomarkers that predict responsiveness to particular treatments are needed, particularly as treatment becomes more specific, such as targeting specific proteins. Patients with early disease appear to decline more rapidly and might show better responses to therapy, whereas currently, the focus of treatment is on patients with late disease who have the most symptoms. If effective and safe anti-inflammatory therapies are developed for COPD, it might 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 of systemic hypertension and hypercholesterolemia, in which treatment is given to prevent future risk.

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