

Smith Seminars
Online Continuing Education
AARC-Approved for 2 CRCE
Mechanisms of COPD

Objectives

Identify the molecular and cellular components in emphysema.

State the differences between COPD and asthma

List the factors of airflow limitations in COPD

Identify the key inflammatory cells

State the role of oxidative stress factors in COPD

List the components acute exacerbation of COPD

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

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

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

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

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

COPD includes chronic obstructive bronchiolitis with fibrosis and obstruction of small airways, and emphysema with enlargement of airspaces and destruction of lung

parenchyma, loss of lung elasticity and closure of small airways. Chronic bronchitis, by contrast, is defined by a productive cough of >3 months duration for more than two successive years; this reflects mucous hypersecretion and is not necessarily associated with airflow limitation. Most patients with COPD have all three pathological mechanisms (chronic obstructive bronchiolitis, emphysema and mucus plugging) as all are induced by smoking, but may differ in the proportion of emphysema and obstructive bronchiolitis. In developed countries cigarette smoking is by far the most common cause of COPD accounting for >95% of cases, but there are several other risk factors, including air pollution, poor diet, and occupational exposure. COPD is characterized by acceleration in the normal decline of lung function seen with age. The slowly progressive airflow limitation leads to disability and premature death and is quite different from the variable airway obstruction and symptoms in asthma, which rarely progress in severity. While COPD and asthma both involve inflammation in the respiratory tract there are marked differences in the nature of the inflammatory process, with differences in inflammatory cells, mediators, response to inflammation, anatomical distribution and response to anti-inflammatory therapy. However, some patients appear to share the characteristics of COPD and asthma. Rather than this representing a graded spectrum of disease, it is more likely that these patients have both of these common diseases at the same time.

Differences from Asthma

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

Predominant Mechanisms of Airflow Limitation

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

Small Airways

There is narrowing of small airways in patients with COPD. There is an increase in the thickness of small airways with increased formation of lymphoid follicles and deposition of collagen in the outer airway wall that may restrict airway opening. The lumen of small airways is reduced by mucosal thickening containing inflammatory exudates, which

increases with the severity of disease. The mechanism for lymphoid follicle formation in more severe disease is unknown, but may reflect a response to chronic bacterial colonization and acute exacerbations of inflammation. The mechanisms of fibrosis around the airway are not yet understood, but are likely to represent an attempt to repair chronic inflammation. The role of specific growth factors, such as transforming growth factor-beta (TGF-beta) which shows increased expression in peripheral airways and connective tissue growth factor (CTGF) are not yet known. TGF-beta may induce fibrosis via the release of CTGF which may stimulate collagen deposition in the airways.

Emphysema

Both panacinar and centrilobular emphysema may occur in smokers. The role of emphysema in causing airflow obstruction in COPD have been examined by measuring macroscopic emphysema in resected lung or on computerized tomography (CT) scans in relation to tests of lung function, or by measuring static transpulmonary pressure (PL) as a measurement of alveolar disease. Many studies have shown significant correlations between the grading of macroscopic emphysema and various tests of lung function. However, the assessment of macroscopic emphysema is dominated by destroyed or poorly functioning lung, whereas lung function tests reflect predominantly the function of the best surviving lung. In the extreme case of non-ventilated emphysematous bullae surrounded by normal lung, the two assessments are virtually independent, with lung function tests measuring only the reduced volume of surviving lung. In more common types of emphysema, a simple two compartment model does not apply, but usually there will be greater heterogeneity of disease with more and more units becoming poorly functional as disease progresses, resulting in a rise in residual volume and a fall in vital capacity.

PL (measured using an esophageal catheter) is plotted against airflow conductance or maximal expiratory flow at different lung volumes to indicate the contribution of alveolar disease (and by implication emphysema) to airflow limitation, with the assumption that the rest is due to intrinsic airway disease. However, it is not certain that the magnitude of decline in PL accurately reflects the severity of emphysema and its effects on the airways. Reduction in PL is likely to be largest with relatively uniform emphysema, as occurs in panacinar emphysema (such as alpha₁-antitrypsin deficiency), whereas patchy centrilobular emphysema may have near normal PL. Uniform microscopic emphysema might account for functional pseudoemphysema without any CT change.

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

increase is all due to intrinsic disease of the peripheral airways underestimates the role of emphysema. Emphysema may play a more prominent role in severe disease as the decline in lung function accelerates.

Mucus Hypersecretion

The contribution of mucus hypersecretion to airflow limitation in COPD is still uncertain. Recent studies have demonstrated that mucus hypersecretion may be a potential risk factor for accelerated decline in lung function. The most likely mechanism whereby chronic mucus hypersecretion contributes to progression of COPD may be due to the increased risk of exacerbations that appear to accelerate loss of forced expiratory volume in one second (FEV1). Chronic mucus hypersecretion may contribute little in the early phases of COPD when exacerbations are infrequent. It is possible that chronic mucus hypersecretion may reflect the inflammatory process around submucosal glands and may reflect the intensity of inflammation in more peripheral airways. Increased numbers of neutrophils and mast cells have also been found around submucosal glands and serine proteases and mast cell chymase are potent mucus secretion stimulators. In severe COPD chronic mucus hypersecretion is associated with mortality and this may also reflect an increased risk of infection. Chronic cough and mucus production in smokers with normal lung function do not appear to predict the later development of COPD.

Key Inflammatory Cells

COPD, like asthma, is a complex inflammatory disease that involves several types of inflammatory cells and multiple inflammatory mediators. However, the pattern of inflammation and the spectrum of mediators differ between these two airway diseases, at least in the stable state of the disease. Although abnormal numbers of inflammatory cells have been documented in COPD, the relationship between these cell types and the sequence of their appearance and their persistence are largely unknown. Most studies have been cross-sectional based on selection of patients with different stages of the disease and comparisons have been made between smokers without airflow limitation (normal smokers) and those with COPD who have smoked a similar amount. Analysis of the cell profile in alveoli and small airways shows an increase in all of the cell types implicated in COPD, including macrophages, T-lymphocytes, B-lymphocytes and neutrophils.

Neutrophils

Increased numbers of activated neutrophils are found in sputum and BAL fluid of patients with COPD, yet there are only relatively small increases in the airways or lung parenchyma. This may reflect their rapid transit through the airways and parenchyma. Neutrophils secrete serine proteases may contribute to alveolar destruction. These serine proteases are also potent mucus stimulants. Neutrophil recruitment to the airways and parenchyma involves adhesion to endothelial cells and E-selectin is upregulated on endothelial cells in the airways of COPD patients. Adherent neutrophils then migrate into the respiratory tract under the direction of neutrophil chemotactic factors. Neutrophil survival in the respiratory tract may be increased by cytokines. The role of neutrophils in COPD is not yet clear. There is a correlation between the number of circulating neutrophils and fall in FEV1. Neutrophil numbers in bronchial

biopsies and induced sputum are correlated with COPD disease severity and with the rate of decline in lung function. Smoking has a direct stimulatory effect on granulocyte production and release from the bone marrow. Smoking may also increase neutrophil retention in the lung. There is no doubt that the neutrophils recruited to the airways of COPD patients are activated as there are increased concentrations of granule proteins in the sputum. These neutrophils also show an increase in the respiratory burst response which correlates with the degree of airflow limitation.

Neutrophils have the capacity to induce tissue damage through the release of serine proteases and oxidants. Priming is a prerequisite for degranulation and superoxide anion generation in neutrophils. Neutrophils in the peripheral circulation show evidence of priming in COPD, but this may result from, rather than contribute to, lung pathophysiology. There are several chemotactic signals that have the potential for neutrophil recruitment in COPD and which are increased in COPD airways. The mediators may be derived from alveolar macrophages and epithelial cells, but the neutrophil itself may be a major source.

Neutrophils from circulation migrate to the pulmonary circulation and adhere to endothelial cells in the alveolar wall before passing into the alveolar space. The precise route for neutrophil migration in large airways is less certain, but it is more likely that they reach the airway from the tracheobronchial circulation and migrate across post-capillary venules. The cellular mechanisms underlying neutrophil adhesion and transmigration differ between systemic and pulmonary circulations and this might confer different properties on the neutrophils arriving from the alveolar or bronchial compartments. There may be significant differences in neutrophil transit times in different areas of the lung that may account for differential distribution of emphysema, for example the upper lobe predominance in centrilobular emphysema. Little is known about survival and apoptosis (cell self-destruction) of neutrophils in COPD airways. Neutrophils have the capacity to cause elastolysis, this is not a prominent feature of other pulmonary diseases where chronic airway neutrophilia is even more prominent, including cystic fibrosis and bronchiectasis. This suggests that other factors are involved in the generation of emphysema. There is a negative association between the number of neutrophils and the amount of alveolar destruction in COPD and neutrophils are not a prominent feature of parenchymal inflammation in COPD. It is likely that airway neutrophilia is linked to mucus hypersecretion in chronic bronchitis. Serine proteases from neutrophils are all potent stimulants of mucus secretion from submucosal glands and goblet cells in the epithelium.

Macrophages

Macrophages appear to play a pivotal role in the pathophysiology of COPD and can account for most of the known features of the disease. They are activated by cigarette smoke extract and secrete many inflammatory proteins that may orchestrate the inflammatory process in COPD. Macrophages generate reactive oxygen species (ROS) and nitric oxide (NO) which together form peroxynitrite and may contribute to steroid resistance.

There is a marked increase (5–10 fold) in the numbers of macrophages in airways, lung parenchyma, BAL fluid and sputum in patients with COPD. An analysis of macrophage numbers in the parenchyma of patients with emphysema showed a 25-fold increase in the

numbers of macrophages in the tissue and alveolar space compared with normal smokers. Macrophages are localized to sites of alveolar wall destruction in patients with emphysema. There is a correlation between macrophage numbers in the airways and the severity of COPD.

Macrophages may be activated by cigarette smoke extract to release inflammatory mediators, providing a cellular mechanism that links smoking with inflammation in COPD. Alveolar macrophages also secrete elastolytic enzymes taken up from neutrophils. 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.

The increased numbers of macrophages in smokers and COPD patients may be due to increased recruitment of monocytes from the circulation in response to monocyte-selective chemokines. The increased numbers of macrophages in COPD may also be due to increased proliferation and prolonged survival in the lungs. Macrophages have a very low proliferation rate in the lungs, but there is some increase in cell proliferation measured by proliferative cell nuclear antigen. Macrophages have a long survival time so this is difficult to measure directly. However in macrophages from smokers, there is markedly increased expression of the anti-apoptotic protein which suggests that macrophages may have a prolonged survival in smokers and patients with COPD.

Corticosteroids are ineffective in suppressing inflammation, including cytokines, chemokines and proteases, in patients with COPD. In vitro the release of macrophages from normal subjects and normal smokers are inhibited by corticosteroids, whereas corticosteroids are ineffective in macrophages from patients with COPD. The reasons for resistance to corticosteroids in COPD and to a lesser extent macrophages from smokers may be the marked reduction in activity of histone deacetylase, which is recruited to activated inflammatory genes by glucocorticoid receptors to switch off inflammatory genes. The reduction in histone deacetylase activity in macrophages is correlated with increased secretion of cytokines and reduced response to corticosteroids. The reduction of histone deacetylase activity on COPD patients may be mediated through oxidative stress and peroxynitrite formation.

Although corticosteroids are not effective in inhibiting the secretion of cytokines and proteases from macrophages, other drugs may be beneficial. Theophylline in low concentrations increases histone deacetylase activity in alveolar macrophages and in vitro reverses the steroid resistance induced by oxidative stress. Resveratrol, a flavenoid found in red wine, is an effective inhibitor of cytokine expression from macrophages from COPD patients, but its molecular mechanisms of action have not yet been determined. Macrophages are phagocytic for bacteria and play an important role in host defense. The phagocytic potential of macrophages from COPD patients has not been explored, but it is possible that impaired phagocytosis may result in the increased bacterial load in the respiratory tract of patients with COPD. Macrophages recognize apoptotic cells via expression of phosphatidylserine which interacts with specific receptors on the macrophage surface. Neutrophil elastase cleaves the phosphatidylserine receptor and may impair the ability of macrophages to take up apoptotic neutrophils, resulting in increased numbers of apoptotic neutrophils in the airways.

T-lymphocytes

There is an increase in the total numbers of T-lymphocytes in lung parenchyma, peripheral and central airways of patients with COPD. There is a correlation between the numbers of T-cells and the amount of alveolar destruction and the severity of airflow obstruction. The mechanisms by which T-cells accumulate in the airways and lungs of patients with COPD is not yet understood. However, homing of T-cells to the lung must depend upon some initial activation then adhesion and selective chemotaxis.

CD8+ cells are typically increased in airway infections and it is possible that the chronic colonization of the lower respiratory tract of COPD patients by bacterial and viral pathogens is responsible for this inflammatory response. It is also possible that protease-induced lung injury may uncover previously sequestered autoantigens or that cigarette smoke itself may damage airway epithelial cells and make them antigenic.

With increased numbers of CD4+ cells in COPD, it is possible that they have immunological memory and play a role in perpetuating the inflammatory process in the absence of cigarette smoking. Natural killer cells are the first line of defense against viral infections. Circulating natural killer cells are reduced in patients with COPD and have reduced phagocytic activity and similar findings are found in normal smokers, although no difference in natural killer cells was found in lung parenchyma of COPD patients.

There is an increase in T-cells in alveoli of smokers, whether they have airway obstruction or not.

The role of T-cells in the pathophysiology of COPD is not yet certain. CD8+ cells have the capacity to cause cytolysis and apoptosis of alveolar epithelial cells. There is an association between CD8+ cells and apoptosis of alveolar cells in emphysema.

Eosinophils

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

Dendritic Cells

The dendritic cell plays a central role in the initiation of the innate and 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, T- and B-lymphocytes. It is therefore likely that the dendritic cell may play an important role in the pulmonary response to cigarette smoke and other inhaled noxious agents and may therefore be a key cellular element in COPD. The mechanism by which tobacco smoke activates the

immune system is not yet understood, but a glycoprotein isolated from tobacco has powerful immuno-stimulatory actions. There is an increase in the number of dendritic cells in rat lungs exposed to cigarette smoke and in the airways and alveolar walls of smokers. Pulmonary histiocytosis is a disease caused by dendritic cell granulomata in the lung and is characterised by destruction of the lung parenchyma that resembles emphysema. The adult form of the disease occurs almost exclusively in smokers. In mice exposed to chronic cigarette smoke there is an increase in dendritic cells in the airways and lung parenchyma.

Dendritic cells may play an important role in the pathophysiology of chronic obstructive pulmonary disease as they can be activated by cigarette smoking and by tobacco glycoprotein resulting in recruitment of neutrophils, macrophages, natural killer cells, CD4+ and CD8+ T-lymphocytes and B-lymphocytes.

Epithelial cells

Airway and alveolar epithelial cells may be an important source of inflammatory mediators and proteases in COPD. Epithelial cells are activated by cigarette smoke to produce inflammatory mediators. Epithelial cells in small airways may be an important source of TGF-beta, which then induces local fibrosis.

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

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

Role of Oxidative Stress

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

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including, neutrophils, eosinophils, macrophages, and epithelial cells. Superoxide anions (O₂⁻) are generated by reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and this is converted to hydrogen peroxide (H₂O₂) by superoxide dismutases. H₂O₂ is then dismutated to water by catalase. Superoxide anions

and H₂O₂ may interact in the presence of free iron to form the highly reactive hydroxyl radical (.OH). Superoxide anions may also combine with nitric oxide 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.

Granulocyte peroxidases, such as myeloperoxidase in neutrophils, play an important role in oxidative stress. In neutrophils H₂O₂ generated from O₂⁻ is metabolised by myeloperoxidase in the presence of chloride ions to hypochlorous acid which is a strong oxidant. Myeloperoxidase is also able to nitrate tyrosine residues, as can peroxynitrite.

Antioxidants

The normal production of oxidants is counteracted by several antioxidant mechanisms in the human respiratory tract. The major intracellular antioxidants in the airways are catalase, superoxide dismutase (SOD) and glutathione, formed by the enzyme gamma-glutamyl cysteine synthetase, and glutathione synthetase. Oxidant stress activates the inducible enzyme heme oxygenase-1 (HO-1), converting heme and hemin to biliverdin with the formation of carbon monoxide (CO). Biliverdin is converted via biliverdin reductase to bilirubin, which is a potential antioxidant. HO-1 is widely expressed in human airways and CO production is increased in COPD. 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. The glutathione system is the major antioxidant mechanism in the airways. There is a high concentration of reduced glutathione in lung epithelial lining fluid and concentrations are increased in cigarette smokers. Extracellular glutathione peroxidase (eGPx) is an important antioxidant in the lungs and may be secreted by epithelial cells and macrophages, particularly in response to cigarette smoke or oxidative stress. eGPx inactivates H₂O₂ and O₂⁻, but may also block reactive nitrogen species. Extracellular antioxidants also include the dietary antioxidants vitamin C and vitamin E, uric acid, lactoferrin and extracellular SOD3. SOD3 is highly expressed in human lung, but its role in COPD is not yet clear.

Effects on Airways

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

ROS activate transcription factor nuclear factor-kappaB (NF-kappaB), which switches on multiple inflammatory genes resulting in amplification of the inflammatory response. The molecular pathways by which oxidative stress activates NF-kappaB have not been fully explained. Many of the stimuli that activate NF-kappaB appear to do so via the formation of ROS, particularly H₂O₂. ROS activate NF-kappaB in an epithelial cell line and increase the release of pro-inflammatory cytokines from cultured human airway epithelial cells. 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. Another transcription factor that activates inflammatory genes is activator protein-1. As with NF-kappaB there are several steps in the activation pathway. Exogenous oxidants may also be important in worsening airway disease. Cigarette smoke, ozone and, to a lesser extent, nitrogen dioxide, impose an oxidative stress on the airways.

Oxidants also activate mitogen-activated protein (MAP) kinase pathways. H₂O₂ is a potent activator of extracellular regulated kinases and p38 MAP kinase pathways that regulate the expression of many inflammatory genes and survival in certain cells, and the spreading of macrophages. Many aspects of macrophage function are regulated by oxidants through the activation of multiple kinase pathways.

Evidence for Increased Oxidative Stress

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

There are several markers of oxidative stress that may be detected in the breath and several studies have demonstrated increased production of oxidants in exhaled air or breath condensates. There is an increased concentration of H₂O₂ in exhaled breath condensate of patients with COPD, particularly during exacerbations.

There is also an increase in the concentration of 8-isoprostaglandin F₂alpha (8-isoprostane) in exhaled breath condensate, which is found even in patients who are ex-smokers and is increased further during acute exacerbations. Isoprostane is also increased in the breath of normal smokers, but to a lesser extent than in COPD, suggesting that there is an exaggeration of oxidative stress in COPD. 8-Isoprostane is similarly increased in the urine of patients with COPD and further increased during exacerbations.

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

The increased oxidative stress in the airways of COPD patient may play an important pathophysiological role in the disease by amplifying the inflammatory response in COPD.

Oxidative stress may also impair the function of antiproteases and accelerate the breakdown of elastin in lung parenchyma.

Corticosteroids are much less effective in COPD than in asthma and do not reduce the progression of the disease. In contrast to patients with asthma, those with COPD do not show any significant anti-inflammatory response to corticosteroids. Alveolar macrophages from patients with COPD show a marked reduction in responsiveness to the anti-inflammatory effects of corticosteroids, compared to cells from normal smokers and

nonsmokers. Recent studies suggest that there may be a link between oxidative stress and the poor response to corticosteroids in COPD. Oxidative stress impairs binding of glucocorticoid receptors to DNA and the translocation of these receptors from the cytoplasm to the nucleus. Corticosteroids switch off inflammatory genes by recruiting histone deacetylase 2 (HDAC2), to the active transcription site and by deacetylating the hyperacetylated histones of the actively transcribing inflammatory gene, they are able to switch off its transcription and thus suppress inflammation. In cigarette smokers and patients with COPD there is a marked reduction in activity of HDAC and reduced expression of HDAC2 in alveolar macrophages and an even greater reduction in HDAC2 expression in peripheral lung tissue. This reduction in HDAC activity is correlated with reduced suppression of inflammatory cytokines and a reduced response to corticosteroids. This may result directly or indirectly from oxidative stress and is mimicked by the effects of H₂O₂ in cell lines.

Oxidative stress may also induce apoptosis in endothelial and epithelial cells. Apoptosis of type 1 pneumocytes may be contributory to the development of emphysema and this might be induced by cytotoxic T-lymphocytes or by inhibition of vascular-endothelial growth factor receptors. ROS may induce apoptosis by activating the NF-kappaB pathway, by direct DNA damage.

Proteases

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

Neutrophil Elastase

There has been particular emphasis on the role of neutrophil elastase since patients with inherited alpha1-antitrypsin deficiency were shown to develop early onset emphysema. Furthermore the demonstration that alpha1-AT may be inactivated by cigarette smoke exposure raised the possibility that neutrophil elastase may also be important in smokers with normal plasma alpha1-AT concentrations. Neutrophil elastase is a serine protease which is inhibited by alpha1-AT in the lung parenchyma.

Neutrophil elastase has been shown to have several other actions relevant to its potential role in COPD. It is a potent mucus secretion stimulator of submucosal gland cells and goblet cells; induces the expression of some cytokines, including IL-8 in airway epithelial cells. Neutrophil elastase cleaves the phosphatidylserine receptor on macrophages, thus impairing their ability to clear apoptotic cells.

On the other hand neutrophil elastase also inactivates a cell surface receptor for lipopolysaccharide, thus reducing the inflammatory response to endotoxin.

The role of neutrophil elastase in COPD will only be established when the effect of neutrophil elastase inhibitors has been studied clinically.

Cysteine Proteases

Lysosomal cysteine proteases (cathepsins) may also be involved in COPD. Cathepsin S expression is induced by interferon-gamma in several cell types, including smooth muscle cells. The role of cathepsins in COPD is uncertain. Increased concentrations of cathepsin L have been detected in BAL fluid of patients with emphysema and alveolar macrophages from patients with COPD secrete more cysteine protease activity than macrophages from normal smokers or nonsmokers. The endogenous inhibitors of cathepsins are cystatins and stefins, but little is known about their role in COPD. Cystatin C concentrations are increased in BAL fluid of patients with COPD.

Matrix Metalloproteinases

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

Antiproteases

Normally proteases are counteracted by an excess of endogenous antiproteases. The major inhibitors of serine proteases are alpha1-AT in lung parenchyma and airway epithelium-derived SLPI in the airways. Serine protease inhibitors inactivate neutrophil elastase and other serine proteases. Multiple genetic variants of alpha1-AT are now recognized that give rise to reduced circulating active alpha1-AT concentrations. SLPI is the other major serine proteinase inhibitor in the airways. SLPI may be inactivated by oxidative stress, but also by cleavage through its active site by cathepsins L and S and in patients with emphysema proteolytic fragments of SLPI are found in BAL fluid which contributes to the reduced anti-neutrophil elastase activity in these patients. This inactivation of SLPI not only impairs its anti-neutrophil elastase activity, but also its antimicrobial and anti-inflammatory roles.

The Amplifying Mechanisms

The inflammatory changes and protease imbalance in COPD are also seen in cigarette smokers without COPD but to a lesser extent, suggesting that the accelerated decline in lung function in COPD may be due to amplification of the normal pulmonary inflammatory response to irritants. This may be due to increased production of inflammatory mediators and enzymes, or because of defective endogenous anti-inflammatory or antiprotease mechanisms. These differences might be explained by

polymorphisms in the genes encoding cytokines, proteases, anti-inflammatory proteins and antiproteases.

Another hypothesis is that these differences are due to latent virus infection. The latent adenovirus sequence E1A is more commonly detected in the lungs of patients with emphysema than in matched smoking control subjects and is correlated with an increased inflammatory response.

Another molecular mechanism that may underlie the amplification of inflammation in COPD may involve impaired activity of histone deacetylase in alveolar macrophages. In macrophages from cigarette smokers there is impaired activity of histone deacetylase, which is involved in switching off the transcription of inflammatory genes by reversing the acetylation of core histones that is associated with their activation. In COPD patients there is even more marked reduction in histone deacetylase activity in the peripheral lung than in smokers without airway obstruction. This may lead to amplification of the expression of inflammatory genes, as is seen in alveolar macrophages from patients with COPD.

Although smoking is the major causal mechanism in COPD, quitting smoking does not appear to result in resolution of the inflammatory response in the airways, particularly in advanced disease. This suggests that there are perpetuating mechanisms that maintain the chronic inflammatory process once it has become established. This may account for presentation of COPD in patients who stopped smoking many years before their first symptoms develop. The mechanisms of disease persistence are currently unknown.

Poor Response to Corticosteroids

Inhaled corticosteroids are now the mainstay of chronic asthma therapy and the recognition that chronic inflammation is also present in COPD provided a rationale for their use in COPD. Inhaled corticosteroids are now widely prescribed in the treatment of COPD and are used almost as frequently as in asthma. However, the inflammation in COPD is not suppressed even by high doses of inhaled or oral corticosteroids. This may reflect the fact that neutrophilic inflammation in humans is not suppressible by corticosteroids as neutrophil survival is prolonged by steroids. Approximately 10% of patients with stable COPD show some symptomatic and objective improvement with oral corticosteroids and it is likely that these patients have concomitant asthma, as both diseases are very common. Airway hyperresponsiveness, a characteristic of asthma, may predict the rate of decline in COPD. A response to corticosteroids is predicted by increased numbers of eosinophils in sputum and an increase in exhaled nitric oxide, both characteristic features of asthma.

Studies have shown that high doses of inhaled corticosteroids fail to reduce the progression of COPD. Inhaled corticosteroids may have a small effect in reducing exacerbations in patients with severe disease.

It seems likely that there is an active resistance to corticosteroids in COPD. High doses of corticosteroids fail to reduce cytokine and chemokines that should be suppressed by corticosteroid treatment. The molecular mechanisms of corticosteroid resistance are not yet known, but may be the same mechanisms that result in amplification of inflammatory responses. Thus a reduction in histone deacetylase activity in macrophages may prevent the anti-inflammatory action of corticosteroids, which is dependent on recruitment of histone deacetylases to the inflammatory gene complex.

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

Mechanisms of Acute Exacerbations

Although acute exacerbations of COPD, defined by increased symptoms and worsening lung function, are a common cause of hospital admission, their cellular and molecular mechanisms are far from clear. Acute exacerbations may be prolonged and have a profound effect on the quality of life and may accelerate the progression of COPD. It was always assumed that the increased amount and purulence of sputum signified bacterial infection of the respiratory tract, but it is now evident that many exacerbations in COPD (as in asthma) are also due to upper respiratory tract viral infections (particularly rhinovirus) and to environmental factors, such as air pollution and temperature. There is an increase in neutrophils and concentrations in sputum during an exacerbation, and patients who have frequent exacerbations have higher levels of IL-6 and lower concentrations of SLPI, even when COPD is stable. There is also an increase in the activation of NF-kappaB in alveolar macrophages during exacerbations of COPD, providing a link between infections, oxidative stress and the enhanced expression of inflammatory genes. Purulent exacerbations are associated with bacterial infection and are characterized by a marked increase in leukotriene (LTB₄) concentrations in sputum. Chronic bacterial colonization of sputum is correlated with increased inflammatory indices in sputum and predisposes to more frequent purulent exacerbations. Bronchial biopsies show an increase in eosinophils during exacerbations in patients with mild chronic bronchitis and this may reflect the marked upregulation of RANTES (regulated on activation, normal T-cell expressed and secreted) seen in airway epithelial cells during acute exacerbations. However, there is no increase in sputum eosinophils during exacerbations in patients with severe COPD. An increase in markers of oxidative stress and exhaled nitric oxide, presumably reflecting increased airway inflammation, are observed during exacerbations. This may reflect increased activation of NF-kappaB during exacerbations. Exacerbations of COPD appear to be due to yet further amplification of the inflammatory process. This implies that treatments that suppress inflammation in COPD would also block exacerbations.

The reason why the lower respiratory tract of some patients with COPD is colonized with bacteria, such as nontypeable strains of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* is uncertain. In a recent study 24% of 600 sputum specimens taken during an acute exacerbation contained a bacterial pathogen compared to 18% inpatients without exacerbations. Approximately one third of exacerbations were associated with "new" strains of bacteria with a differing protein composition, suggesting newly-acquired infection rather than a flare-up of existing organisms. However these "new" strains might arise from antigenic variants of persistent colonizing strains, which may make them less susceptible to local immune mechanisms. Persistence of certain bacteria in the lower respiratory tract of COPD patients may be due to localization to protected sites. *H. influenzae* is localized to sites within the airway wall, such as between cells in the airway walls and these organisms are not susceptible to antibiotics or

antibody-mediated defense mechanisms when they are located between airway epithelial cells. *H. influenzae* infection is associated with increased concentrations of inflammatory mediators in COPD patients. The inflammatory process itself may also promote persistence of certain bacteria. For example, human neutrophil defensins may increase adhesion of *H. influenzae* to airway epithelial cells and may thus predispose to invasion.

Conclusion

The understanding of the cellular and molecular mechanisms involved in COPD is at an early stage compared with knowledge of asthma. Although both diseases involve inflammation in the respiratory tract, the pattern of inflammation, the results of the inflammatory process and the therapeutic response are markedly different.

Although activation of several inflammatory cells has now been identified in COPD, their relative importance and sequential role in producing the typical pathology of COPD are still poorly understood. However, it is likely that cigarette smoke and other irritants activate resident cells, including macrophages, epithelial cells and dendritic cells, which then signal the influx of other inflammatory cells, including neutrophils, monocytes and T-lymphocytes from the circulation. These cells all release multiple mediators of inflammation, although the pattern of mediators differs from those found in asthma. The pathological process is predominantly located in the lung periphery with involvement of small airways and lung parenchyma. However, the relationship between inflammation and fibrosis in small airways and destruction of lung parenchyma and mucus hypersecretion are uncertain and there are differences in the preponderance of these mechanisms between patients and at different stages of the disease.

There appears to be an amplification of the inflammatory process between smokers who do not have airflow limitation and the minority of smokers with accelerated decline of lung function who develop COPD. The mechanisms of amplification may be linked to the relative steroid resistance found in COPD and a plausible link is deficiency in Histone deacetylase activation that both amplifies inflammatory gene expression and impairs the anti-inflammatory response to corticosteroids.

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