Interrelated role of cigarette smoking, oxidative stress, and immune response in COPD and corresponding treatments

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Zuo L, He F, Sergakis GG, Koozehchian MS, Stimpfl JN, Rong Y, Diaz PT, Best TM. Interrelated role of cigarette smoking, oxidative stress, and immune response in COPD and corresponding treatments. *Am J Physiol Lung Cell Mol Physiol* 307: L205–L218, 2014. First published May 30, 2014; doi:10.1152/ajplung.00330.2013.—Cigarette smoking (CS) can impact the immune system and induce pulmonary disorders such as chronic obstructive pulmonary disease (COPD), which is currently the fourth leading cause of chronic morbidity and mortality worldwide. Accordingly, the most significant risk factor associated with COPD is exposure to cigarette smoke. The purpose of the present study is to provide an updated overview of the literature regarding the effect of CS on the immune system and lungs, the mechanism of CS-induced COPD and oxidative stress, as well as the available and potential treatment options for CS-induced COPD. An extensive literature search was conducted on the PubMed/Medline databases to review current COPD treatment research, available in the English language, dating from 1976 to 2014. Studies have investigated the mechanism by which CS elicits detrimental effects on the immune system and pulmonary function through the use of human and animal subjects. A strong relationship among continued tobacco use, oxidative stress, and exacerbation of COPD symptoms is frequently observed in COPD subjects. In addition, therapeutic approaches emphasizing smoking cessation have been developed, incorporating counseling and nicotine replacement therapy. However, the inability to reverse COPD progression establishes the need for improved preventative and therapeutic strategies, such as a combination of intensive smoking cessation treatment and pharmaceutical therapy, focusing on immune homeostasis and redox balance. CS initiates a complex interplay between oxidative stress and the immune response in COPD. Therefore, multiple approaches such as smoking cessation, counseling, and pharmaceutical therapies targeting inflammation and oxidative stress are recommended for COPD treatment.

reactive oxygen species; inflammation; antioxidant; smoking cessation

CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as "a common preventable and treatable disease," demonstrating "persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases" (102a). This disorder is characterized by a decrease in both maximum expiratory flow and forced expiratory volume in 1 s (FEV_1). Exposure to noxious particles through continued cigarette smoking (CS) associated with increased oxidative stress may result in frequent hospitalization, decreased quality of life, as well as reduced productivity (78, 80, 99, 136). Smokers with COPD typically consume more tobacco than average and exhibit an increased nicotine dependence (63). As a result, continued tobacco use can lead to millions of deaths and billions of dollars in estimated healthcare costs. Accordingly, an estimated 90% of all deaths from COPD can be attributed to CS (124). Research has shown that early smoking cessation alleviates the progression of this debilitating pulmonary disease (8, 9, 107). However, the symptoms of COPD are not resolved after smoking cessation, indicating the involve-

ment of other endogenous factors, such as increased oxidative stress and impaired immunity (30, 52).

In general, patients with CS-induced COPD have a reduced FEV_1 combined with elevated oxidative stress (78, 80, 136). Yet, little is known about the mechanisms associated with these risk factors. Wang et al. (134) indicated that the components contained within cigarette smoke extract (CSE), acetaldehyde, and acrolein, as well as the nonvolatile components contained within lyophilized CSE could inhibit human airway epithelial cell chemotaxis. These results suggest that CS may cause the pathophysiological disruptions observed in the airways of individuals with COPD.

The close relationship among continued tobacco use by individuals with COPD, worsening COPD health outcomes, and the documented benefits of quitting necessitates a careful examination of the evidence regarding treatment strategies. A literature search through the use of the PubMed/Medline database was conducted to review studies from the years 1986 to 2014 that pertain to the adverse effects of tobacco use on the immune system, CS-induced COPD, tobacco treatments of COPD patients, and other therapeutic approaches. This is a review of clinical relevance that employs a multisearch approach using various key words including "tobacco," "COPD," "oxidative stress," "smoking cessation," "pharmacotherapy for nicotine dependence," "nicotine replacement therapy," and "COPD treatment." Moreover, potential therapeutic approaches targeting cellular and molecular mechanisms of COPD pathogenesis and exacerbations are also discussed. In summary, this review aims to provide an update on the interrelated roles of CS, the immune response, and the oxidative stress in COPD pathogenesis, as well as discuss conventional and other potential treatment strategies for this disease.

Effects of Tobacco on the Immune System in COPD

Studies have indicated that the antigenic chemicals in cigarettes can lead to the development of antigen-antibody complexes, which may potentially induce pulmonary and peripheral injuries (11). CS is associated with the release of proinflammatory cytokines, the activation of inflammatory cells, and the restriction of anti-inflammatory mediators (Table 1). CS-induced systemic inflammation is characterized by an increase in circulating inflammatory molecules, such as acute-phase proteins and proinflammatory cytokines (141). Several studies have reported that CS can cause elevated levels of TNF-α, TNF-α receptors, interleukin (IL)-1, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (17, 27, 42). Particularly, a women's health study conducted in the United States showed a trend of increasing IL-6 levels across non-, former, and current women smokers (17). Furthermore, Wirtz

et al. (138) observed a trend of higher baseline TNF-α levels among smokers.

In support of these observations, serial analysis of gene expression and comparative microarray analysis revealed that inflammatory cytokines (i.e., TNF-α and other interleukins) and chemokine-related genes were significantly upregulated in COPD subjects (87). In general, the increased levels of cytokines and/or chemokines released by inflammatory cells after cigarette smoke exposure serve as chemoattractants, recruiting more neutrophils, macrophages, and dendritic cells, which, in turn, exacerbate the inflammatory process. The newly recruited inflammatory cells continue the inflammatory process through phagocytosis, secretion of cytokines, and the initiation of both reactive oxygen species (ROS) and surface antigen formation to mediate innate and adaptive immune responses (36, 70).

Cigarette smoke comprises more than 4,500 components in its gaseous and particulate phases. These compounds include direct carcinogens (methylcholanthrene, benzo-α-pyrenes, and acrolein), toxins (carbon monoxide, nicotine, ammonia, acetone, and hydroquinone), reactive solids with chemically catalytic surfaces, and oxidants (superoxide and nitrogen oxides) (117). In addition, the immune system of a smoker is less efficient in combatting bacterial infection compared with that of a nonsmoker (120); in fact, bacterial infection may exacerbate COPD symptoms (102). Particularly, CS increases the amount of polymorphonuclear neutrophils (PMNs; the most abundant white blood cells), while decreasing their functionality, such as phagocytic and antimicrobial activity involved in the first lines of host defense. The systemic inflammatory response elicited by cigarette smoke exposure is determined by the stimulation of the hematopoietic system, which is attributed to the elevation of PMN counts in smokers (113). A study of circulating PMNs with phenotypic alterations indicated that smoking elicits an accelerated passage of PMNs from the bone marrow into the circulatory system (131). The elevated levels of circulating PMNs in smokers (up to 30% compared with nonsmokers) results mainly from nicotine-induced secretion of catecholamines (12).

Furthermore, in vitro exposure of PMNs to CSE induces prominent morphological and morphometric changes, as well as metabolic alterations (143). It has been shown that high concentrations of nicotine inhibit antimicrobial functions (88, 140), resulting in decreased superoxide production by blood PMNs. This observation is consistent with Sørensen et al. (114), who demonstrated that the oxidative burst of PMNs and monocytes were 50 and 68% less, respectively, in smokers compared with nonsmokers. Similar to PMNs, macrophages are extensively distributed immune cells that have a crucial role in homeostasis and defense. Because of their antigen-

Table 1. *Effect of CS on inflammatory markers*

Cytokine/Chemokine	Function	Main Effect of CS on Cytokine/Chemokine
TNF-α	Initiation and preservation of inflammation, activation of endothelial and epithelial cells	Increased by CS (12, 27, 42)
IL-1	Influence transcription; mediate, in part, the pathogenesis of disease	Increased by CS (6, 12, 27)
IL-6	Multifunctional cytokine (inflammation, vascular permeability, cell proliferation)	Increased by CS (12, 17, 141)
IL-8	A potent leukocyte chemotactic factor, specific to neutrophils	Increased by CS (12, 34)
IL-10	Suppression of T helper cells (type 1 and 2) responses, stimulation of B cells and antigen-specific cytotoxic T cells	Decreased by CS (12)

CS, cigarette smoking. Reference numbers are shown in parentheses.

presenting function and phagocytic properties, macrophages are the primary cell population that serves as the first line of defense against pollutants (67). This being said, chronic smoke exposure can result in an influx of alveolar macrophages into the lumen of the airway, initiating inflammation (54). In addition, CS induces increased DNA damage in alveolar macrophages, and inhibits the expression of CD11b, TLR-2, and CD14, which are important surface antigens associated with phagocytic activity, ultimately resulting in significant impairment of alveolar macrophage function. Therefore, continued CS increases the susceptibility of COPD subjects to bacterial and/or viral infections (50).

Although the mechanisms of CS-induced lung damage have not been fully elucidated, it has been proposed that early inflammatory responses are responsible for tissue damage (18, 20). Studies have shown that CS may result in a localized reaction and sequestration of neutrophils via the release of toxic and chemotactic mediators in pulmonary microvasculature, further predisposing the lungs to inflammatory disease (18, 20). For example, it has been found that the population of inflammatory cells recovered from bronchoalveolar lavage fluid (BALF) is higher in smokers compared with nonsmokers (34). In smokers, an increase in the number of inflammatory cells in sputum and an increase in the amount of small airway macrophages was also observed (5).

It is well established that the innate immune system, consisting of macrophages, neutrophils, natural killer cells, and dendritic cells, is essential in the initiation and progression of COPD. Recently, Gadgil and Duncan (40) thoroughly examined the role of adaptive immune cells, namely T cells, in the development of COPD. Specifically, $CD8^+$ T cell-derived mediators predominately contribute to inflammation, alveolar wall destruction, and small airway fibrosis in the later stages of COPD (40). This is consistent with the higher levels of neutrophils and lymphocytes observed in smokers (5, 24, 34, 104). Since COPD is a progressive disorder associated with an abnormal lung inflammatory response to noxious particles or gases (96), inflammation normally occurs as a result of $CD8^+$ T cells in the peripheral and central airways and the lung parenchyma. This being said, an increase in $CD8^+$ T cells, and consequently a decrease in the $CD4^+/CD8^+$ T cell ratio, have been frequently observed in the airway submucosa of smokers (71). Therefore, an elevated $CD8^+$ T cell count may be used as a marker to distinguish between smokers with and without COPD (15). The highly activated T cells in COPD patients are also associated with a broad array of Th1 chemokines and cytokines (44, 72). The overall inflammatory process is derived from the release of antigens caused by the breakdown of elastin. These antigens in turn are relayed to the Th1 cells, which play an important role in the later stages of COPD (44, 72).

Furthermore, Churg et al. (25) postulated about the mechanism of the CS-induced inflammatory matrix and severe COPD conditions such as emphysema. As a stimulus, cigarette smoke causes chemokine/cytokine release from alveolar macrophages, epithelial cells, and fibroblasts. This results in an influx of neutrophils (e.g., PMNs), macrophages, lymphocytes, and dendritic cells as well as proteases [such as neutrophil elastase and macrophage-derived matrix metalloproteinase (MMP)-12], which can directly attack the alveolar wall matrix, leading to the release of elastin fragments, chemoattractants that further

exacerbate inflammation and lung damage in emphysema (25). Accordingly, MMPs are secreted by inflammatory cells and may alter the physiological morphology of the lung parenchyma. MMPs are commonly activated by interaction with other proteases, which causes degradation of the alveolar wall matrix and incites the progression of COPD. Proteases associated with CS-induced COPD may also facilitate the release of membrane-bound TNF-α, similar to the function of TNF-α converting enzyme (TACE) (28, 65). In addition, MMP-12 is commonly secreted by macrophages and plays an important role in the pathogenesis of COPD (28, 116). In a study by Churg et al. (28), it was shown that, in contrast to control mice, MMP-12-deficient mice did not exhibit an increase in whole lung TNF-α levels following cigarette smoke exposure, indicating that MMP-12 may correlate to a TNF-α-induced inflammatory response. Elevated levels of MMP-1, MMP-2, and MMP-9 have been observed in the lung tissue of COPD subjects (58, 74). Moreover, researchers have found that inhalation of cigarette smoke has an equal effect on the concentration of MMP-1 in both COPD patients and healthy individuals (28, 116). MMP-12 and MMP-1 also play a critical role in airway remodeling as a means to combat CS-induced inflammation. Thus numerous recent studies have emphasized the important role of the immune response in the pathogenesis of COPD.

Role of Reactive Oxygen Species in COPD

Previous research has shown increased oxidative stress and escalated catalase activity during low-oxygen conditions, as well as in asthma and COPD subjects (136, 145, 147, 149). This enhanced antioxidant enzymatic activity is an indirect result of oxidative stress induced by COPD (136). Accordingly, COPD patients exhibited increased exhaled ROS (7, 128). Moreover, numerous studies have indicated greater levels of oxidative stress in cigarette smokers (78, 80), which is most likely attributed to the high concentration of ROS in cigarette smoke (25). In addition, CS has been associated with elevated ROS production by leukocytes in the airways of both acute and chronic smokers (21). Subsequently, this production is stimulated by the release of ROS by neutrophils and macrophages, in conjunction with the inflammatory response (such as TNF-α production) to CS (78, 146). Furthermore, excess CS-induced ROS cannot be neutralized by endogenous antioxidants, ultimately resulting in oxidative stress-induced damage (21), which has been frequently demonstrated in animal models. Aoshiba et al. (10) indicated that cigarette smoke exposure imposes oxidative stress primarily on bronchiolar epithelial and alveolar type II cells. Rangasamy et al. (98) also reported an upregulation of a variety of antioxidant enzymes after mice were exposed to cigarette smoke. Interestingly, Harju et al. (46) observed that the expression of superoxide dismutase (SOD) (an antioxidant enzyme) is higher in the central bronchial and alveolar epithelium of smokers with COPD than in that of nonsmokers, demonstrating the active involvement of antioxidants during oxidative stress.

Although the exact etiology of COPD pathogenesis is not clear, increased levels of ROS, overwhelming the endogenous antioxidant defense, is likely one of the major driving mechanisms. Understanding the source of ROS production in COPD and the role of elevated oxidative stress in the exacerbation of

COPD is critical for the development of novel therapeutic approaches. Cigarette smoke is the predominant extraneous source of oxidative stress, consisting of high concentrations of oxidants (35, 93, 94), as shown in Table 2. Moreover, levels of endogenous ROS (i.e., superoxide leakage from complex I and III) produced by mitochondrial respiration in airway epithelial and smooth muscle cells (4, 130), cytoplasmic-generated ROS (i.e., NADPH oxidase and the xanthine oxidase in BALF), and myeloperoxidase (MPO) released from activated PMNs and macrophages during oxidative burst, are markedly elevated during COPD exacerbation, even after CS cessation (1, 75, 92). A putative mechanism of CS-induced oxidative stress in the pathogenesis of COPD was proposed by Barnes in 2013 (13). In the mechanism, ROS activate NF-κB and p38 mitogen-activated protein kinase (MAPK), leading to the activation of proinflammatory/inflammatory cytokine and chemokine genes. Additionally, ROS contribute to the imbalance of endogenous proteases/antiproteases leading to increased elastolysis, accelerating damage to the lung. Excessive ROS production results in DNA damage, lipid peroxidation, and protein carbonylation of airway endothelial and alveolar cells, aggravating the symptoms of COPD (148).

Effects of CS in Animal Models

COPD is an irreversible inflammatory disease that progressively worsens in the absence of appropriate treatments (90). Although the devastating impact of tobacco on human health is well known, a detailed mechanism involved in the pathogenesis of COPD has yet to be determined. Animal models of COPD may provide insight into the cellular and molecular mechanisms of CS-induced COPD, as well as advance our understanding of CS, oxidative stress, and the immune response as it relates to the specific pathways of COPD pathogenesis.

Churg et al. in 2008 (25) reviewed the mechanism of CS-induced COPD in animal models. The researchers suggested that antiproteases may be utilized to initiate anti-inflammatory intervention (e.g., TNF-α inhibitor). They also suggested that antioxidant approaches appear to be promising therapeutic strategies to attenuate emphysema in CS-induced COPD animal models. Kang et al. (66) demonstrated that cigarette smoke could enhance airway and alveolar inflammation and apoptosis, as well as exaggerated emphysema and airway fibrosis. It was concluded that the mechanism of these augmented inflammatory and remodeling responses is mediated by the MAVS/PKR/IL-18Rα/interferon (IFN)-γ pathway. Several other studies (16, 48) also confirm this enhanced inflammatory response in a CS-induced COPD mouse model. Although innate immunity plays an important role in COPD exacerbation-, CS- or virus-induced inflammatory and remodeling responses, the mechanisms that mediate these responses have not been completely defined.

Recently, Nikota and Stampfli (86) suggested that CS disrupts lung immune homeostasis (i.e., exacerbates the inflammatory responses and skews the respiratory host defense) in an animal model and that the resulting altered immune responses likely contribute to the pathogenesis of COPD. A detailed understanding of the inflammatory mechanism triggered by microbial infections and exacerbated by CS may potentially provide a novel intervention in COPD treatment. However, because of ethical considerations, there are limited clinical studies investigating the mechanisms involved; animal research (e.g., mouse, guinea pig, rat) has been proven advantageous in the exploration of the mechanisms involved in CS-induced COPD (15). In addition, recent studies (14, 61, 82) have demonstrated that CS can impair the antiviral host defense. For instance, type I IFN, a key mediator involved in antimicrobial signaling pathways, is attenuated after cigarette smoke exposure. It is well established that IFR (type I interferon receptor) also plays a pivotal role in antiviral activities. Thus excessive IFR degradation contributes to immune suppression under viral or bacterial infection. Interestingly, these impaired host defense responses elicited by chronic smoke exposure are reversed by antioxidants such as reduced glutathione (GSH), N-acetylcysteine (NAC), and others via the suppression of IFR degradation (55, 97).

Autoimmunity appears to be another novel aspect that may contribute to the pathogenesis of COPD and emphysema (12, 72). However, studies focusing on the mechanism of the adaptive immune response (i.e., subsets of T cells, B cells, and mast cells) and the autoimmune response in COPD have been largely overlooked. It is speculated that chronic CS could accelerate the progression of emphysema through the disruption of lung immune homeostasis and host defenses. Thus more in-depth studies are needed in this area. Overall, animal studies have illustrated that both chronic inflammation and the innate immune response play central roles in the pathogenesis of CS-induced COPD.

CS, Oxidative Stress, and Immune Response in COPD

Barnes (13) recently reviewed new anti-inflammatory targets for COPD, illustrating the relationship between oxidative stress, the immune response and the pathogenesis of CS-induced COPD. Specifically, cigarette smoke activates patho-

Table 2. *Oxidants, antioxidants, and their function*

Oxidant/Antioxidant	Function	Main Effect of CS on Oxidant/Antioxidant
GSH	Involved in the defense against ROS	Decreased by acute CS; increased by chronic CS (78)
TEAC	Scavenges free radicals and other potentially toxic oxidizing species	Decreased by CS (79)
8-Isoprostane	Serves as a marker of oxidative stress; involved in the pathogenesis of atherosclerosis as well as other inflammatory disorders	Increased by CS (80)
Nitrosothiol	Serves as a donor of nitrosonium and nitric oxide	Increased by CS (80)
eNO	An antioxidant; mediates vascular tone; nonadrenergic and noncholinergic regulation of the inflammatory response	Increased by CS (7, 57)

Reference numbers are shown in parentheses. eNO, exhaled nitric oxide; GSH, reduced glutathione; ROS, reactive oxygen species; TEAC, trolox equivalent antioxidant capacity.

gen-associated molecular patterns (PAMPS) in macrophage and epithelial cells in the respiratory tract, which in turn, activate Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors. Together with CS-induced ROS, these receptors ultimately trigger proinflammatory cytokine and chemokine (e.g., NF-κB, IL-1β, IL-8) generation. These released chemokines, serving as chemoattractants, induce both innate and adaptive immune responses by recruiting more neutrophils, monocytes, macrophages, and T lymphocytes. Released proteases can also break down connective tissues in the lung, potentially resulting in chronic bronchitis or emphysema. In addition, activated macrophages or neutrophils with increased MPO activity attributed to CS-induced chemotactic factors can release multiple proinflammatory mediators that exacerbate COPD inflammation.

It is clear that elevated oxidative stress linked to phagocytosis is important for removing damaged tissue and eradicating invading microbes. However, excessive ROS released during phagocytosis via oxidative bursts of PMNs and alveolar macrophages have been shown to inhibit antiprotease processes and accelerate the degradation of lung tissue. ROS also delay the resolution of inflammation via compromising the phagocytotic ability of alveolar macrophages, leading to necrosis and emphysema. Likewise, long-term cigarette smoke exposure impairs PMN and alveolar macrophage phagocytosis and antigen-presentation functions, which could predispose patients to bacterial/viral infection. Bacterial colonization in the lower respiratory tract due to CS, combined with compromised host defenses, as well as chronic inflammation, could explain the exacerbation of COPD even after smoking cessation.

Collectively, CS-stimulated dysregulation of the immune response together with the imbalance of oxidant production and antioxidant defense contributes to the development and the progression of COPD. Treatments targeting key components (stimulus, proinflammatory mediators, redox-sensitive molecules, etc.) of the cellular and molecular pathway of COPD pathogenesis should be well investigated in the development of successful therapeutics.

Tobacco Treatment Strategies in COPD-Targeting Stimulus

Benefits of quitting. A seminal study by Anthonisen and colleagues (8, 9), conducted in 1994 (with a long-term follow-up conducted 14.5 years later), called the Lung Health Study (LHS), demonstrated that tobacco interventions significantly reduced FEV_1. The LHS, a 5-year early intervention study, examined 3,926 smokers with mild-to-moderate airflow limitation and compared usual care to intensive smoking cessation counseling and nicotine replacement therapy. The LHS was the first randomized clinical trial to report that an aggressive smoking intervention program significantly reduces the age-related decline in FEV_1 in smokers that remain abstinent. Additionally, other researchers studied LHS data to examine lung function decline in intermittent smokers and smokers that simply reduced the number of cigarettes smoked per day. The loss of lung function in these two subgroups was noted as similar to that of the continued smokers (56, 112). The findings of these studies underscore the importance and benefits of complete and sustained tobacco cessation in reducing COPD morbidity and mortality.

Although tobacco use has declined in the U.S. in the past 50 years since the first Surgeon General's report regarding the dangers of tobacco in 1964, more than 43.8 million adults continue to use tobacco. The majority of adult tobacco users want to quit (68.8%), but very few succeed without intervention (22a). Fortunately, effective treatments are available. In 2008, the U.S. Public Health Service (PHS) commissioned the Tobacco Use and Dependence Guideline Panel to update the clinical practice guideline on tobacco treatment. The PHS guideline synthesized 8,700 research articles, all of which further investigated the use of evidence-based strategies for smokers attempting tobacco cessation (37). Many of these strategies were also proven to be effective for smokers with COPD. Moreover, multiple studies have been conducted to examine the tobacco treatment strategies for such smokers with COPD. Similarly, meta-analyses and reviews often compare the effectiveness of pharmacological, nonpharmacological, and combination interventions for this population of smokers.

Pharmacotherapy. Throughout the world, CS is the primary risk factor for lung cancer, cardiovascular disease, and COPD. The use of pharmacotherapy, specifically nicotine replacement therapy (NRT), addresses the discomfort of withdrawal experienced from tobacco cessation. There are seven first-line pharmacotherapies recommended in the Clinical Practice Guideline (37). These include the nonnicotine medications bupropion and varenicline and the NRT medications gum, patch, lozenge, inhaler, and nasal spray. In COPD, the use of pharmacotherapy for treatment of tobacco dependence increases abstinence rates greater than nonpharmacological treatment alone (126). Pharmacotherapy has been shown to be cost effective and beneficial in increasing long-term cessation in smokers with COPD (53, 123, 126). The use of NRT and other pharmacotherapy has also been shown to immensely increase abstinence rates in healthy smokers (111). Tonnesen et al. (126) explored the long-term efficacy of sublingual NRT for cessation in COPD smokers and found that cessation rates were similar to those of healthy smokers. The use of pharmacotherapy in COPD smokers is recommended to include NRT, bupropion, or varenicline, singly or in combination, at standard or high doses. For a prolonged period up to 12 mo, NRT pharmacotherapy has been proven to assist the smoker with COPD to quit (62). These studies found this to be an effective method of smoking cessation and the results reflect those found with healthy smokers.

Non-NRT pharmacotherapy has also been studied among COPD smokers. Tashkin and colleagues (123) suggested that bupropion is a well-tolerated and effective treatment strategy for smokers with mild to moderate COPD. Jimenez Ruiz et al. (64) studied 472 patients with severe or very severe COPD (GOLD stages III and IV) and found continuous abstinence rates from 9 to 24 wk as high as 58.3% with use of varenicline. The same study found that varenicline and bupropion yield higher abstinence rates than NRT. They concluded that varenicline was more effective than nicotine patches, 61 vs. 44% (odds ratio: 1.98; 95% confidence interval: 1.25–3.12; $P = 0.003$). Moreover, another pharmacological treatment that may become available in the future is the nicotine vaccine for smoking cessation. Evidence regarding the effectiveness of the vaccine continues to amass in Phase II and III clinical trials (47).

Nonpharmacological treatment. Nonpharmacological strategies for tobacco treatment include simple advice, written self-help materials, formal supports (individual or group), and telephone quit lines (125). To support smoking cessation, several studies have examined the use of communicating abnormal spirometry results or discussing "lung age" with smokers as a "teachable moment" (69, 89). The results of such studies are inconclusive. Physician advice (118), nurse-facilitated support groups (137), and support groups for hospitalized COPD patients (19) are reported to be only partially effective.

Combined approaches. Evidence suggests that multicomponent cessation programs wherein health care providers combine counseling with pharmacotherapy, ongoing support, and follow-up when needed can further improve cessation rates. It was reported that counseling, pharmacotherapy treatments, and NRT are also effective (53, 119, 124, 127, 133). According to two independent systematic reviews by Warnier et al. (135) and van der Meer et al. (129), pharmacological and behavioral (psychosocial) intervention was more effective than no intervention. Furthermore, both of the reviews concluded that a combination of pharmacological and behavioral (psychosocial) intervention was more effective than each alone. Table 3 reviews the combination approaches studied and relates the abstinence rates reported for each strategy.

Antioxidant Therapeutic Strategies in COPD-Targeting Oxidative Stress

It is well established that increased oxidative stress from the oxidants found in tobacco smoke as well as the ROS produced during chronic inflammatory responses contributes to the pathogenesis and progression of COPD. Antioxidants and free radical-scavenging compounds may provide directed therapeutics against this oxidative stress and subsequent tissue damage. NAC, a thiol antioxidant that serves as a precursor for GSH (a powerful endogenous antioxidant), is able to attenuate elastase-induced pulmonary emphysema in rats (105). However, recently, a large randomized placebo-controlled clinical trial investigated the outcomes of NAC treatment in 523 patients with COPD and concluded that NAC failed to prevent lung dysfunction (indicated by FEV_1) (31). The poor outcomes of the study indicate that single antioxidant treatment may not be effective in slowing the exacerbation of COPD. Perhaps the increased bioavailability of GSH might not reach specific components of target cells that require high antioxidant protection. Thus a potential therapeutic strategy has been focused on nuclear factor erythroid 2-derived factor 2 (NRF2). This transcription factor has a pivotal role in the regulation of genes that encode multiple antioxidant proteins (e.g., GSH) and is found to be significantly declined in patients with COPD (81).

Antioxidants such as NAC, Mito-TEMPO, and SOD have also been observed to alleviate mitochondrial fragmentation and apoptosis, as well as reduce cellular senescence and the opening of mitochondrial permeability transition pores (45, 73). Although the use of antioxidants in the treatment of COPD is limited, investigations underlying the therapeutic index of radical-scavenging pharmaceuticals are currently underway. It was recently reported that through the reduction of superoxide to hydrogen peroxide, lecithinized SOD (PC-SOD) can effectively mitigate distorted lung mechanics and elastase- and CS-induced inflammation. For this reason, PC-SOD has been suggested as a promising therapeutic option to increase airway resistance in emphysema (121).

Certain cruciferous plants, especially broccoli, are becoming of increasing interest in COPD because of their powerful influence on the endogenous redox processes. Not only do these plants contain isothiocyanates and glucosinolates, valuable cytoprotective and anticarcinogenic compounds (32), but they also contain natural antioxidants, folate, vitamin C, and β-carotene. A recent pilot study of the effects of broccoli intake in male smokers determined that the components of broccoli may upregulate plasma antioxidants, as well as influence glutathione *S*-transferase glutathione activity and enhance innate cellular defenses against oxidative stress-induced DNA lesions (101). In addition, sulforaphane extracted from cruciferous vegetables may protect and increase the activity of NRF2, which in turn may directly augment the expression of downstream antioxidants in COPD subjects (ClinicalTrials.gov identifier: NCT01335971) (13). Thus a balanced diet consisting of fruits and vegetables is essential to maintain the endogenous oxidant/antioxidant balance. Other promising approaches such as celastrol (extracted from the medicinal plant *Tripterygium wildordii*) have been shown to inhibit NADPH oxidase (NOX) in in vitro studies (60). However, animal-based studies and clinical trials in patients with COPD are required to evaluate the effectiveness of this agent. MPO derived from neutrophils and macrophages during oxidative burst can further amplify

Table 3. *Smoking cessation strategies for COPD*

Intervention	Comparison	No. of Studies	Abstinence Rate Pooled Relative Risk (95% CI)	Grade
Counseling	Usual Care	2	5.85 (3.81–8.97)*	Moderate
Intensive counseling ≥90 min	Usual Care	1	7.70 (4.64–12.79)*	Moderate
Minimal counseling <90 min	Usual Care	1	1.56 (0.65–3.72)	Moderate
Counseling + NRT	Usual Care	3	4.28 (3.51–5.20)*	Moderate
Intensive counseling ≥90 min + NRT	Usual Care	1	4.41 (3.60–5.39)*	Moderate
Minimal counseling <90 min + NRT	Usual Care	2	2.11 (0.90–4.91)	Moderate
Minimal counseling <90 min + Antidepressant	Usual Care	1	1.91 (0.65–5.61)	Low
Minimal counseling <90 min + NRT + Antidepressant	Usual Care	1	2.25 (0.87–5.85)	Low
NRT	Placebo	1	3.01 (1.02–8.89)*	Moderate
Antidepressant	Placebo†	2	2.09 (1.35–3.24)*	Moderate
Nortriptyline	Placebo	1	2.54 (0.87–7.44)	Moderate
Bupropion	Placebo	2	2.01 (1.24–3.24)*	Moderate

CI, confidence interval; NRT, nicotine replacement therapy. *Statistically significant ($P < 0.05$). †One trial used in this comparison had 2 treatment arms each examining a different antidepressant. From Thabane M and COPD Working Group (125).

oxidative damage and the inflammation of lung tissue. MPO inhibitor was also reported to mitigate the progression of emphysema following long-term cigarette smoke exposure in animal models (26). Further exploration through clinical trials is recommended to test the effectiveness of MPO inhibitors in COPD.

Overall, antioxidant therapy in patients with COPD is a promising area for future research. Current inconclusive results may be due to a lack in the complete understanding of pharmacokinetics, bioavailability, toxicity, and absorption of various exogenous antioxidants and activators of endogenous antioxidants in these clinical trials. Additionally, the limitation of animal studies (e.g., the lack of COPD exacerbation model, anatomical and physical difference, genetic variance, and varied responses to injury) makes clinical translation difficult (139). Furthermore, targeting endogenous redox-sensitive transcription factors (i.e., NRF2), upstream mediators in the CS-initiated oxidative stress mechanism, and chronic oxidative stress induced by specific cellular components may provide valuable, therapeutic approaches for the mitigation of COPD.

Anti-Inflammatory Therapeutic Strategies-Targeting Immune Homeostasis

For many decades, bronchodilators (e.g., theophylline), serving as cyclic nucleotide phosphodiesterase (PDE) inhibitors, have been widely used to treat mild-to-severe COPD. However, this treatment is not frequently recommended because of its side effects and nonselective nature (39). Drugs specifically targeting phosphodiesterase, such as PDE4 inhibitor (roflumilast), have been shown to improve lung function in patients with COPD. PDE4 inhibitor attenuates the inflammatory response by decreasing the number of neutrophils and CD4+ and CD8+ T cells and inhibiting monocyte chemotaxis activation (49). In addition, corticosteroids, another class of anti-inflammatory agents, are effective in mitigating acute COPD exacerbation; yet corticosteroid resistance has been observed in some patients with stable COPD (43). Additionally, oxidative stress has been found to posttranslationally modify histone deacetylase 2 (HDAC2), leading to its reduction in the lungs of COPD patients (142). Researchers have reported that these chronically decreased levels of HDAC2 can prevent the nuclear translocation of glucocorticoid receptors, which leads to an impaired anti-inflammatory gene expression induced by corticosteroids in patients with COPD (2, 59). Therefore, the reversal of glucocorticoid sensitivity may provide another promising strategy to alleviate COPD-induced inflammatory responses in the future (59).

Furthermore, Cosio et al. (29) and Ford et al. (38) suggested that a low dosage of theophylline combined with corticosteroids is effective in reducing inflammatory cells via an increase in HDAC2 expression in patients with COPD. Multiple kinases are fully engaged in the regulation of proinflammatory transcription factors and inflammatory genes. However, poor selectivity to specific targets may lead to undesirable clinical outcomes (13). Other research has shown that several inhaled selective p38 mitogen-activated protein kinase (p38MAPK) inhibitors (i.e., PF-03715455 and GSK610677) may be promising therapies (84).

As discussed earlier, increased inflammation contributes to COPD, and altered immune homeostasis is the main mecha-

nism leading to emphysema. Furthermore, impaired phagocytosis of apoptotic material by airway macrophages and increased bacterial colonization results in chronic inflammation and COPD exacerbation (41, 100). Interestingly, lower-dose azithromycin was reported to improve bacterial phagocytosis by both alveolar and monocyte-derived macrophages in COPD subjects (51), suggesting that long-term use of low azithromycin doses may be a promising adjunct treatment for COPD. Moreover, potential drugs targeting inflammatory mediators (e.g., TLR inhibitor, chemokine receptor antagonist, TNF antibodies, and IL-17 antibodies) and proteases (e.g., small-molecule neutrophil elastase inhibitor, MMP-9 and MMP-12 inhibitors) encompass a logical therapeutic approach. However, more clinical trials are needed to evaluate the efficacy of these newly developed drugs (22).

Key Studies and Recent Advances: a Literature Review From 2012 to 2013

The noxious components of cigarette smoke elicit chronic lung disease through a variety of complex mechanisms, including redox signaling. Oxidative stress results from a systemic overabundance of ROS that both overwhelm natural host defenses against oxidative insult and elicit the lung epithelial and alveolar cell destruction characteristic of many chronic respiratory diseases including COPD. In COPD, oxidative stress may have a role in the remodeling of the lung parenchyma. Airway remodeling, a functional marker of COPD, has recently been traced to epithelial-mesenchymal transition (EMT) through the nicotine-initiated activation of the Wnt3a cascade and the resulting upregulation of both Wnt and β-catenin signaling in the lung epithelium (144). Although the exact signaling mechanism by which nicotine induces EMT is unclear, nicotine has been proven, through the use of human bronchial epithelial cell (HBEC) lines, to induce β-catenin/Tcf-dependent transcription and thereafter provoke the transdifferentiation of epithelial cells into mesenchymal cells (144). TGF-β$_1$, a mediator involved in profibrotic growth, has also been observed at higher levels in COPD subjects and smokers than in nonsmokers, providing a seemingly direct connection between reactive components of cigarette smoke and airway remodeling in COPD (144). Thus these transdifferentiated pluripotent mesenchymal cells may be a major source of fibrogenic cell establishment, which ultimately contribute to the observed airway remodeling in COPD. Furthermore, transforming growth factor-β-activated kinase 1 (TAK1) has an important role in the activation of MAP kinase and nuclear factor-κB (NF-κB), as well as chemokine release. By pretreating airway smooth muscle cells with TAK1 and NF-κB and ERK-1/2 signaling pathway inhibitors, CSE-induced IL-8 release can be inhibited (91), providing a potential treatment to inhibit CS-induced inflammatory responses. Recent evidence also demonstrates the association between the deletion of IL-17A and a decrease in CS-induced inflammation and alveolar type II cell apoptosis, suggesting that IL-17A also contributes to CS-induced immune responses and its targeted deletion could pose as a novel COPD therapy (23).

More precisely, CS-induced oxidative stress has been correlated with the disruption of selective ion channels within the lung epithelium (33, 95). These ion channels are vital for certain cellular processes and when obstructed, may markedly

enhance chronic lung inflammation. Specifically, the hindrance of a chloride and bicarbonate channel associated with the cystic fibrosis transmembrane conductance regulator (CFTR) gene is associated with difficulties in mucus transport, poor airway hydration, and disease susceptibility in COPD (95). Furthermore, ROS may interfere with the oxidative regulation of amiloride- and ROS-sensitive epithelial sodium channels (ENaC), responsible for the reabsorption of sodium and water (33). ENaC activity is regulated by the production of NOX, which is the rate-limiting factor in edema resolution in the lung; any ROS-induced inhibition of ion channel activity may drastically impede fluid clearance, heighten the inflammatory response, and disrupt the endothelial cell barrier (33).

In addition to oxidative stress-induced disruption of cellular transport, the function of certain cellular structures, namely the epithelial barrier, endoplasmic reticulum, and mitochondria, is impaired by excess redox signaling in CS-induced COPD (45, 122). Current, translational investigations of idiopathic pulmonary fibrosis have provided emerging evidence relating CS to endoplasmic reticulum (ER) stress and subsequent activation of the unfolded protein response in epithelial cells (122). ER stress also is tied to EMT, fibrosis, progressive inflammation, impaired resolution of injury, and accelerated alveolar epithelial cell apoptosis (122). Moreover, a study by Hara et al. (45) evaluated the detrimental effects of CS-derived ROS on mitochondrial fragmentation and cellular senescence. It was observed that cigarette smoke exposure diminished mitochondrial membrane potential of the swollen and fragmented mitochondria, through the knockdown of fusion proteins such as OPA1 and MFNs, suggesting that CS may induce mitochondrial dysfunction (45). Considering that heightened levels of ROS-induced lipid and protein alterations have been previously attributed to cellular senescence in the pathogenesis of COPD, it was concluded that mitochondrial fragmentation due to CS may even further enhance this cellular "aging" and augment disease progression (45). Schweitzer et al. (108) reported that the soluble components of cigarette smoke may directly disrupt the endothelial barrier via the upregulation of neutral sphingo-myelinase-mediated ceramide and the activation of Rho kinase and oxidative stress-dependent p38 MAPK and JNK pathways in response to CS. It was also observed that GSH modulators had the ability to correct these disruptive effects. GSH depletion in alveolar epithelial cells is known to result from CS; evidence also indicates that resveratrol induces GSH synthesis and protects epithelial cells by reversing CSE-induced post-translational modifications of nuclear factor (erythroid-derived 2)-like 2, a transcriptional regulator of antioxidant genes. These results suggest that a dietary supplement of antioxidants could provide a potential novel treatment for COPD (68).

Epithelial and endothelial cells are especially susceptible to oxidative and inflammatory insults because of their large surface area within the lung parenchyma, high blood supply, and exposure to atmospheric gases and pollutants. Accordingly, alveolar epithelial cell inflammation and apoptosis are significant mechanisms in the pathobiology of CS-induced COPD (23, 110). The deleterious components of cigarette smoke recruit macrophages, neutrophils, and T lymphocytes, which, in turn, aggravate air space enlargement and alveolar destruction, culminating in emphysematous inflammation (23). Although the exact role of the innate immune response in COPD is not well known, notable CS-induced, proinflammatory me-

diators are the focus of current research and present potential therapeutic targets in COPD. IL-6, associated with emphysema, is known to bind to the gp130 coreceptor and hyperactivate the transcription of signal transducer and activator of transcription 3 (Stat3) (106). Research has concluded that IL-6 induces lung inflammatory processes by way of Stat3 and promotes emphysema through the upregulation of alveolar cell apoptosis independent of Stat3 in emphysema and COPD, thereby suggesting that IL-6 trans-signaling and Stat3 hyper-activation may provide potential disease biomarkers and therapeutic targets (106). Additionally, a recent study has provided evidence for the initiation of the p38 MAPK and MAPK-activated kinase-2 (MK2) phosphorylation pathways by the CS-induced translation of IL-8 mRNA in bronchial smooth muscle cells (BSMC) (85). This being said, because IL-8 is a chemoattractant, it is suggested that CS may directly recruit neutrophils and propagate inflammation in BSMC by this mechanism. Current research proposes that established CD8 cells in the lungs of COPD subjects also enhance the production of IFN-γ (115). IFN-γ may be a useful indicator of COPD severity and, through mechanisms relatively unknown, play a role in initiating the immune response during virus-induced exacerbation. However, most importantly, through the stimulation of CD40, an important TNF receptor, IFN-γ has been thought to induce the Fas-apoptotic pathway in epithelial cells and stimulate the enlargement of emphysematous airways (110). In addition, extracellular adenosine is often increased during tissue injury and inflammatory insults by the stimulation of ectonucleotidases, CD39 and CD73, or by variations in the activity and expression of adenosine deaminase (76). Recent research has shown that sustained, elevated adenosine exposure may contribute to CS-induced lung endothelial cell apoptosis and emphysema through the activation of p38 and JNK in mitochondria and eventually mitochondrial defects that also lead to cell apoptosis (77).

As previously mentioned, early inflammation is associated with the accumulation of oxidative stress-induced PMN. Sharma et al. (109) found that CSE inhibits platelet-activating factor (PAF) acetylhydrolase activity, which enhances PAF production and PMN adherence. Similarly, Meyer et al. (83) showed that the expression of secretory leukoprotease inhibitor (SLPI) is regulated by Stat1, and both are increased in nasal epithelial cells and BALF of smokers, indicating that altered SLPI regulation and activity induced by CS could play a role in the development of respiratory inflammation and infection.

ROS have been found to interfere with quaternary and tertiary protein structure, disrupt the lipid bilayer, and denature DNA (103, 132). These warped, misfolded, and dysfunctional proteins are normally cleared by a ubiquitin proteasome system, which is the part of the cell's natural recycling machinery. However, recent research has observed heightened levels of polyubiquitinated proteins in the lung parenchyma of smokers, proposing that defective proteasomal protein regulation is associated with CS-induced oxidative stress and COPD conditions (132). Providing that proteasome expression has been known to be downregulated in COPD, new insight postulates that proteasome function is inhibited site specifically in the absence of transcriptional regulation in response to CS. These results present a seemingly direct correlation between impaired proteasome function and CS. The same study also suggested that chymotrypsin-like proteasome activity, essential for the

breakdown of peptides, is somewhat impaired by CS and may result in a distorted protein milieu, eliciting inappropriate immune function of major histocompatibility complex class I molecules (132). Thus even the most acute exposure to the free radical and electrophilic components of cigarette smoke may stimulate redox-sensitive transcription, cellular signaling, metabolic activity, and mitochondrial function, independent of the innate immune response (3).

According to a study by Agarwal et al. (3), CS-induced oxidant-antioxidant imbalance may directly induce low energy metabolism and lead to inflammation and poor lung function associated with COPD. This may be accomplished, in part, by the upregulation of genes involved in cytosolic and mitochondrial redox regulation, oxidative phosphorylation, cellular metabolism, and the F_1 component of mitochondrial synthase in response to CS; additional upregulation of mitochondrial transport and fusion genes also suggest marked advances in mitochondrial activity (3). Following exposure, CS-initiated glutathionylation prompts the downregulation and inhibition of GAPDH, signifying a decreased rate of glycolysis and promotion of the pentose phosphate pathway and glucose-6-phosphate dehydrogenase (3). It is suspected that the lipophilic components of cigarette smoke, including phenols, aldehydes, and polycyclic aromatic hydrocarbons, may play a major role in this reduction of metabolic activity in the mitochondria because of the ease at which they penetrate epithelial cell membranes and disrupt electron transfer chains involved in ATP generation. Physiologically, these alterations in oxidative metabolism induced by CS may impede the clearance of amines, influence epithelial permeability, and further enhance the oxidative stress response (3). Ultimately, CS-induced oxidative stress and redox signaling is fundamental in various mechanisms that result in the pathology and adverse symptoms of COPD.

Summary

CS causes 90% of all cases of COPD, which is defined as "a common preventable and treatable disease," by the Global Initiative for Chronic Obstructive Lung Disease. In addition to its effects on the immune system, CS also induces oxidative damage on cells due to the high levels of ROS both contained in cigarette smoke and elicited by immune cells in conjunction with the natural inflammatory response. Furthermore, CS-induced COPD mouse models provide a potential approach to further understand the mechanism of smoking in COPD pathophysiology. Altered immune homeostasis (e.g., increased pro-inflammatory response and impaired innate and adaptive immune response) accompanied with chronically increased oxidative stress underlies one of the major mechanisms involved in CS-induced COPD (Fig. 1). Understanding the cellular and molecular mechanisms of the pathogenesis and progression of COPD is indispensable to the development of effective therapeutic interventions.

CS cessation is a useful strategy to reduce mortality and morbidity caused by COPD. Treatments for quitting include pharmacotherapy (NRT and nonnicotine medications) and nonpharmacological treatments (psychosocial or behavioral intervention). Interestingly, studies have concluded that a combination of pharmacotherapy, NRT, and nonpharmacological treatment was more effective than either treatment alone (53, 119, 124, 127, 133) and is effective for both COPD and healthy smokers in the general population. According to this evidence-based review, both the disturbance of immune homeostasis (increased inflammation and impaired host defense in the respiratory tract) and the chronic, accumulated oxidative stress elicited by CS substantially contribute to the development and progression of COPD even after smoking cessation. Although potential therapeutic strategies targeting oxidative stress and
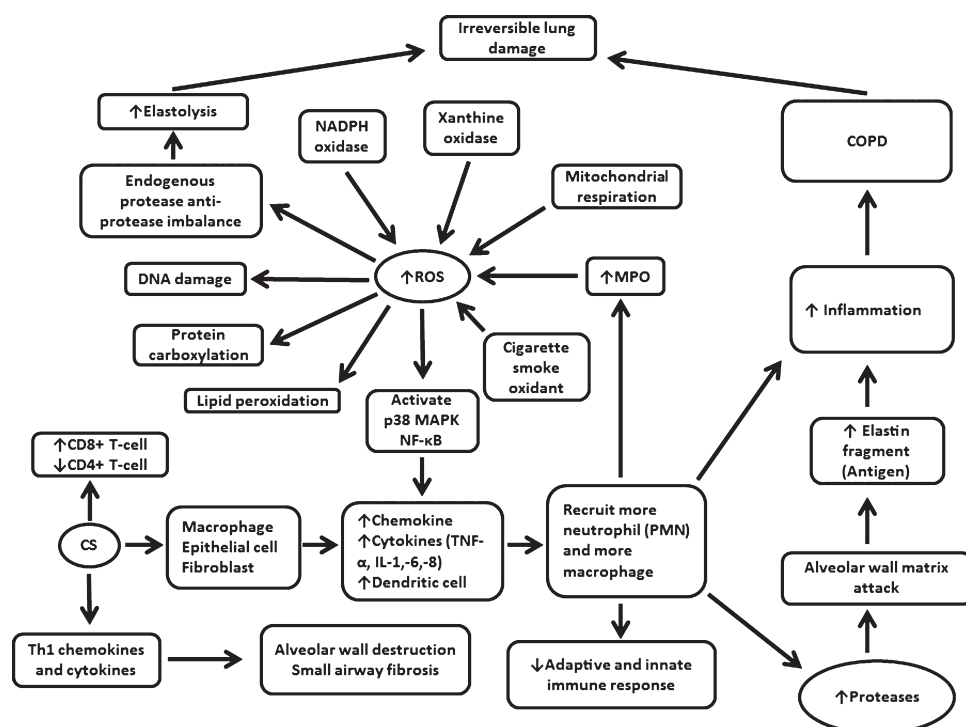
Fig. 1. Proposed mechanism for cigarette-smoking, oxidative stress and the immune response in chronic obstructive pulmonary disease (COPD). CS, cigarette smoking; MPO, myeloperoxidase; p38 MAPK, p38 mitogen-activated protein kinase; PMN, polymorphonuclear neutrophils; ROS, reactive oxygen species.

inflammation have been shown to be promising, to date, there is no cure to reverse or completely halt COPD progression. Thus more systematic studies of CS-induced COPD as well as corresponding treatment strategies are needed. In conclusion, a more advanced understanding of the molecular pathways that contribute to cellular and molecular disease pathogenesis has been developed in recent years; multidisciplinary approaches, including counseling, smoking cessation, the combination of directed anti-inflammatory, antioxidant, and antimicrobial drugs, will provide future directions for COPD- and therapeutic-based research.

Perspectives and Significance

CS is the primary risk factor contributing to the pathogenesis and development of COPD; because of the devastating nature of this disorder, cigarette smokers may be encouraged to seek NRT and nonpharmacological treatments to aid in smoking cessation. Animal model-based studies have provided considerable insight into the mechanism of COPD after cigarette smoke exposure. However, models mimicking the early stages of small airway diseases as well as exacerbation after CS cessation are lacking (13). Therefore, establishing models (not limited to murine) of the different stages and phenotypes of COPD may be essential in the translation of basic research into promising clinical trials. Because of the complexity of the cellular and molecular signaling pathways (e.g., a variety of oxidative stress source, multilevels of proinflammatory mediators, etc.) involved in the pathogenesis of COPD, multidisciplinary therapeutic approaches are necessary to treat this disease. Potential approaches include antioxidants (NRT2 activator), kinase inhibitors (e.g., p38 MARK inhibitor), and drugs targeting the epigenetic regulation of inflammatory responses and the reversal of corticosteroid resistance (i.e., theophylline), as well as other antimicrobials. Finally, inflammatory and redox status varies in cells and tissues at the different stages of COPD. Also, genetic susceptibility to CS-induced COPD also varies with each individual. This being said, the current lack of selectivity and bioavailability of drugs and treatment options is an issue for many patients. Therefore, development of new therapeutic strategies (e.g., small-molecule drugs) with the ability to infiltrate specific cell compartments may aid in the lessening of COPD morbidity and mortality and improve patient quality of life.

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