CHEST Translating Basic Research Into Clinical Practice

Oxidative Stress in COPD

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Oxidative stress is now recognized as a major predisposing factor in the pathogenesis of COPD. Existing therapies for COPD are ineffective at halting disease progression, with bronchodilators being the mainstay of pharmacotherapy, providing symptomatic relief only. It is, therefore, important for a better understanding of the underlying mechanisms by which oxidative stress drives disease pathogenesis to develop novel and more effective therapies. Antioxidant capacity in COPD is substantially reduced as a result of cigarette smoking and exacerbations, with oxidative stress persisting long after the cessation of cigarette smoking or exacerbation, due to the continued production of reactive oxygen species from endogenous sources. We discuss (1) how oxidative stress arises in the lung, (2) how it is neutralized, (3) what genetic factors may predispose to the development of COPD, and (4) how this impacts inflammation and autoimmunity in the development of emphysema and small airways disease. Finally, various strategies have been considered to neutralize the increased oxidative burden present in COPD. This review highlights why current antioxidant strategies have so far failed and what promising alternatives are on the horizon. Moreover, a number of studies have shown that there is no single "magic bullet" to combat oxidative stress, but instead a combination therapy, targeting oxidative stress in the various subcellular compartments, may prove to be more effective in COPD. ***CHEST 2013; 144(1):266–273***

Abbreviations: GSH = reduced glutathione; GST = glutathione-S-transferase; HDAC = histone deacetylase; MDA = malondialdehyde; MPO = myeloperoxidase; NF-κB = nuclear factor-κB; NOX = NADPH oxidase; Nrf2 = nuclear erythroid-2-related factor 2; ROS = reactive oxygen species; SOD = superoxide dismutase

COPD is a major and increasing global health problem that is set to become the third leading cause of death worldwide by 2020. It currently affects about 10% of the population over 45 years of age, rising to 50% in heavy smokers.[1] The major etiologic factor driving this disease is likely to be oxidative and carbonyl stress in the lungs following long-term exposure to cigarette smoke or the combustion products of biomass fuels.[2] Oxidative stress arises as a result of endogenous antioxidant defenses being genetically impaired and/or overwhelmed by the presence of reactive oxygen species (ROS). This in turn can lead to carbonyl stress, where oxidative damage to the surrounding tissues leads to the formation of highly reactive organic molecules that can modify proteins nonenzymatically. COPD is characterized by chronic inflammation and remodeling of the small airways and destruction of the lung parenchyma (emphysema).[3] A striking feature of COPD is its failure to resolve when exposure to cigarette smoke has stopped,[4] which has led to the suggestion that other endogenous factors, such as autoimmunity or persistent infection may also be driving the disease.[1,5]

Manuscript received October 30, 2012; revision accepted January 2, 2013.

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DOI: 10.1378/chest.12-2664

Persistent Lung and Systemic Oxidative Stress in COPD

There is evidence for oxidative and carbonyl stress in COPD, particularly during acute exacerbations. Alveolar macrophages from patients with COPD are more activated and release increased amounts of ROS in the form of the superoxide radical and hydrogen peroxide.[6] Similarly, activated peripheral blood neutrophils from patients with COPD release increased amounts of ROS, particularly during exacerbations. Markers of oxidative stress and carbonyl stress in COPD include elevated concentrations of nitrotyrosine[7]

and lipid peroxidation products, such as 8-isoprostane, 4-hydroxy-2-nonenal, and malondialdehyde (MDA).[8,9] In contrast, concentrations of the endogenous antioxidant glutathione are lower in BAL fluid from patients with COPD with frequent exacerbations compared with those with stable COPD.[10] Although more refined noninvasive methods of assessing oxidative stress have been developed, they are limited due to a lack of standardization.[11]

Despite this, several markers of oxidative stress, for example, hydrogen peroxide, carbon monoxide, myeloperoxidase (MPO),[11,12] and markers of oxidative tissue damage, such as 8-isoprostane[13] and carbonyl stress in the form of MDA,[14] have consistently been shown to be elevated in exhaled breath or exhaled breath condensate from patients with COPD. Moreover, systemic exposure to oxidative stress in COPD is also indicated by increased carbonyl adducts, such as 4-hydroxynonenal in respiratory[8] and skeletal muscle.[15]

Source of ROS in the Lung

The lung is particularly vulnerable to injury from environmental oxidative stress due in part to its anatomic structure. It is constantly exposed to sources of endogenous oxidative stress generated by mitochondrial respiration and inflammatory responses to bacterial and viral infections within the lung. The environmental sources of airborne oxidative stress include oxidant gases and ultrafine particulate material and nanoparticles from industrial pollution and car exhaust fumes. However, the single most important etiologic factor in causing COPD in the western world is cigarette smoking, with inhalation of combustion products from enclosed cooking fires being an important additional etiologic factor in developing countries.[16]

While exposure to cigarette smoke can drive the onset of COPD, once the disease has become established cessation of smoking does not stop the continued presence of oxidative stress and progression of disease.[17] The continued presence of oxidative stress most likely arises from endogenous sources such as mitochondrial respiration. Indeed, airway epithelial cells when exposed to carbonyl stress induce the production of mitochondria-derived ROS,[18] and airway smooth muscle cells from patients with COPD produce greater amounts of mitochondrial-derived ROS when subject to inflammatory stress from IL-1, tumor necrosis factor α, and interferon γ. Pathway analysis has identified mitochondrial dysfunction around complexes I and III as being tightly associated with COPD.[19] In addition, other sources of intracellular ROS include the cytoplasmic ROS-generating enzymes, such as NADPH oxidase (NOX) and the xanthine/xanthine oxidase system as well as the heme peroxidases, levels of which are elevated in broncholavage fluid and inflammatory cells within the airways of patients with COPD.[20,21]

The abundantly produced superoxide radical is a relatively weak oxidizing agent but is the precursor for other more damaging ROS species (Fig 1), such as the hydroxyl radical which is elevated in COPD,[22] or the very powerful and damaging peroxynitrite radical formed by the rapid reaction of superoxide with nitric oxide.[23] Similarly MPO, released from activated neutrophils which accumulate in the lungs of patients with COPD, produces very destructive hypochlorous acid. However, in healthy cells intracellular antioxidant defenses are able to efficiently mop up these ROS species, thus limiting their impact.

Carbonyl Stress in COPD

ROS generation has been directly linked to oxidation of proteins, lipids, carbohydrates, and DNA. The major outcome is the formation of reactive carbonyls and their reaction with proteins, otherwise known as protein carbonylation. This accumulation of reactive carbonyls and subsequent protein carbonylation has been commonly referred to as "carbonyl stress," predominantly associated with chronic disease[24] and aging. Unlike other posttranslational modifications, protein carbonylation is nonenzymatic and targets specific peptide residues, such as lysine, arginine, cysteine, and histidine.

Protein carbonylation is increasingly recognized as a major driver of the underlying pathology associated with many chronic diseases.[25] It is present in both smokers and patients with COPD.[26] Increased levels of free carbonyls, such as MDA, a major product of lipid peroxidation, have also been detected in the lungs of patients with COPD.[9] Levels of carbonyl stress are correlated with disease severity as measured by the decline in FEV_1.[8] Like many posttranslational protein modifications, protein carbonylation can modify protein function, disrupting normal cell function and physiologic mechanisms.[27]

Antioxidant Defenses in the Lung

Because the lung is constantly exposed to both external and endogenous sources of oxidative stress, it has evolved a number of efficient antioxidant defensive strategies, of which reduced glutathione (GSH) plays an important part. Moreover, up to 20% of all glutathione produced is found within the mitochondria to neutralize endogenous ROS production as a by-product of metabolism.[28] Protecting the exposed surface of the lung from the environment is the epithelial lining fluid, which contains several antioxidants that include

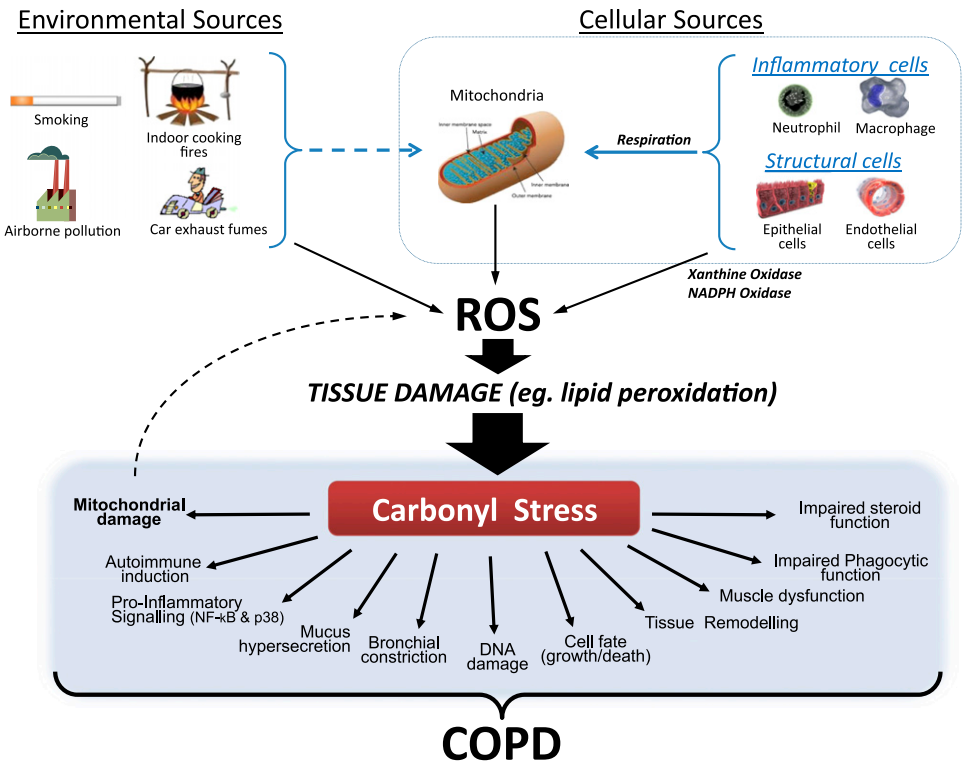

FIGURE 1. A mechanism for the development of COPD driven by oxidative stress through the formation of carbonyl stress. Oxidative stress from environmental and cellular sources causes tissue damage through lipid peroxidation and the oxidation of proteins and carbohydrates resulting in the formation of carbonyl stress. Carbonyl stress, in turn, causes nonenzymatic posttranslational modifications on proteins that can alter protein function, as well as result in the formation of danger-associated molecular patterns (DAMPs) and neo-autoantigens. Importantly, damage to mitochondrial proteins by carbonyl stress only helps to drive further endogenous ROS production by the damaged mitochondria. Together, these carbonyl-modified proteins help to drive the pathophysiologic mechanisms associated with the development of COPD. NF-κB = nuclear factor-κB; ROS = reactive oxygen species.

ascorbic acid (vitamin C), α-tocopherol (vitamin E), and uric acid. Larger molecules such as, albumin and mucin, can also act as sacrificial antioxidants due to the presence of exposed sulfydryl groups. Several studies have shown a clear association between reduced levels of the antioxidants in the lung, such as α-tocopherol and ascorbic acid, and deteriorating pulmonary function in COPD. This, however, may simply reflect an increased oxidative burden as a result of repeated exacerbations. No studies to date have shown that dietary supplementation with antioxidants leads to clinical improvement.[29] However, a 10-year follow-up study did find that antioxidant supplementation reduced the risk of developing chronic lung disease by 10%[30] and lowered carbonyl stress levels in the lung.[31]

The exposure of airway epithelial cells from healthy subjects to acute oxidative stress triggers increased GSH synthesis by upregulating the expression and activity of a key enzyme in GSH synthesis, glutamylcysteine ligase.[32] However, the amount of this enzyme is lowered around the central bronchial epithelium and in alveolar macrophages from smokers and patients with COPD,[33] suggesting a defective regulatory mechanism. Similar differential responses between subjects with COPD and control subjects were apparent with other GSH-dependent antioxidant enzymes, glutathione-S-transferase (GST) pi isoenzyme, GST M1, and glutathione peroxidase.[34] A genetic deletion mutation in GST M1 is associated with the development of emphysema in smokers and increased susceptibility to developing COPD.[35] Similarly, genetic polymorphisms in the GST pi isoenzyme have been associated with COPD.[36]

Transforming growth factor-β (TGF-β) expression is increased in COPD and inhibits the expression of the antioxidant enzymes catalase and superoxide dismutase 2 (SOD2), also known as manganese-SOD, in airway smooth muscle cells.[37] Both these enzymes, which are critical for neutralizing mitochondrial-derived ROS, are under the control of the transcription factor FOXO3. Moreover, a deficiency in FOXO3 activity has previously been associated with COPD.[38] Gene polymorphisms for SOD2 have also been shown to be highly associated with COPD,[39] although few data are available to show how these polymorphisms

equate to changes in functional activity. Similarly, polymorphisms in SOD3 (extracellular SOD) have also been linked to both reduced lung function in COPD[40] and protection against the development of COPD in smokers when SOD3 activity is enhanced.[41] Over 200 cellular antioxidant and detoxification enzymes are under the control of the transcription factor nuclear erythroid-2-related factor 2 (Nrf2), which regulates gene expression through binding to antioxidant response elements within the promoters of the many antioxidant and cytoprotective genes.[42] Patients with COPD have reduced expression of Nrf2-responsive genes due to reduced Nrf2 activity.[43] Upregulation or restoration of Nrf2 activity may, therefore, prove to be of therapeutic benefit in COPD.[44]

Oxidative Stress and Inflammation in the Airways

At least 50 different cytokines and chemokines have been found to be associated with COPD. Many of the intracellular signaling pathways triggered and/or driving the release of these inflammatory mediators are sensitive to oxidative stress as they incorporate redox-sensitive molecular targets, such as the transcription factor nuclear factor-κB (NF-κB) and signaling molecules such as Ras/Rac, Jun-N-terminal kinase, p38 mitogen-activated protein kinase, and protein tyrosine phosphatases. Oxidative stress can activate the NF-κB pathway at many levels and NF-κB expression and activation is increased in COPD and correlates with airflow limitation.[45] Moreover, ROS also act as intracellular second messengers, as inflammatory stimuli induce microoxidative bursts which are essential for cellular activation.[46] Carbonyl stress in the form of electrophilic carbonyls can also impact on many different signaling pathways.[47] As with oxidative stress, this is propagated through the targeting of critical cysteine residues in susceptible signaling molecules.[47]

Resolution of the inflammatory response is equally as important as its induction and the clearance of apoptotic cells by phagocytosis plays a major role in this process. Phagocytosis is impaired in COPD[48] and a failure to remove apoptotic cells can lead to secondary necrosis and continued inflammation in COPD.[49] The impact of oxidative/carbonyl stress on phagocytosis would appear multifactorial with the effects being both intracellular and extracellular. Intracellularly, oxidative stress activates RhoA impairing phagocytosis through changes in cytoskeletal reorganization.[50] Extracellularly, oxidative/carbonyl stress results in carbonylation of tissue proteins creating competition for the same pattern recognition receptors (PRRs) expressed on alveolar macrophages that recognize and clear both carbonyl-modified protein and apoptotic cells.[51] These PRRs necessary for phagocytosis have themselves been shown to be carbonyl modified and thereby impaired.[52] The ability of corticosteroids to repress proinflammatory gene expression is also impaired in COPD as a result of oxidative stress.[53] Carbonylation and nitration reduce the activity and expression of an important transcriptional corepressor histone deacetylase 2 (HDAC2), which is essential for the suppression of activated inflammatory genes and the antiinflammatory actions of corticosteroids.[54,55] Moreover, loss of HDAC2 activity, as observed in COPD,[56] has also been demonstrated to lead to a loss of Nrf2 activity through increased Nrf2 acetylation thereby decreasing Nrf2 stability and expression.[43] This leads to an interesting paradox whereby oxidative/carbonyl stress will activate Nrf2 inducing the expression of protective antioxidant defenses, but chronic exposure to oxidative/carbonyl stress can inhibit/reduce the effectiveness of Nrf2 activation by reducing HDAC2 activity. Indeed, oxidative stress activates the enzyme phosphoinositide-3-kinase-δ, which is also responsible for reducing HDAC2 activity and expression.[57] Another transcriptional corepressor, sirtuin-1, is similarly impacted by oxidative stress, reducing both its expression and activity leading to an accelerated aging process[58] and the increased likelihood of developing emphysema as the lung ages more rapidly.[59] Oxidative stress can, thus, result in enhanced inflammatory gene expression, failure to resolve the inflammatory response, corticosteroid insensitivity, a decreased capacity to induce endogenous antioxidant defenses, and a rapidly aging lung in COPD with increased risk of developing emphysema.

Oxidative Stress and Autoimmunity in COPD

Accumulating evidence has shown that there is an autoimmune component in COPD.[60] Until recently, a mechanistic link between exposure to oxidative stress and developing autoimmunity in COPD was not established. However, autoantibodies against carbonyl-modified self-proteins, as a result of oxidative stress, are elevated in COPD serum and increase with disease severity. Since these autoantibodies are complement fixing, they could contribute to parenchymal lung destruction.[26] Carbonyl-modified proteins are highly immunogenic and can result in autoimmunity.[61] Carbonyl-modified proteins are recognized by the innate immune system through PRRs that are expressed on antigen-presenting cells, such as macrophages and dendritic cells,[62,63] whereupon these potent immunogens are processed and reexpressed in association with major histocompatibility complex II, thereby facilitating the activation of an acquired immune response. Indeed, patients with COPD exhibit a strong type 1 immune response in

the lower airways with the pulmonary accumulation of Th1 cells[1] and dendritic cells in the small airways of patients with COPD,[64] expressing increased amounts of major histocompatibility complex II. It is not clear, however, whether this autoantibody response to oxidatively-modified protein epitopes in COPD is destructive, protective, or simply a bystander effect. However, the autoantibodies against carbonyl-modified protein were of a potentially destructive IgG1 isotype[26] and evidence of corresponding IgG and complement (C3) deposition has been observed in COPD.[26,65]

Besides oxidative stress creating the essential neoantigens, it also helps to drive the influx of immune cells necessary to recognize and process these neoantigens. Increased oxidative stress in the lungs causes the release of CCL20 and CCL2 which in turn triggers the recruitment of dendritic cells, monocytes, and lymphocytes. Helping to orchestrate this immune response in COPD are elevated levels of IL-17 and IL-18,[66,67] which are important for the activation and maturation of B cells and promoting an autoimmune response. IL-18 promotes IL-17 expression and oxidative stress has been demonstrated to activate IL-18 signaling pathways with attenuation of IL-18 preventing further lung destruction.[68]

Therapeutic Implications

There are currently no treatments that reverse or even slow the progression of COPD. Inhaled corticosteroids are highly effective in reducing the inflammatory component in asthma, but provide little therapeutic benefit in COPD. While they may have a small effect in reducing exacerbation frequency, they fail to reduce the inflammatory component and halt the inexorable decline in lung function. This resistance can be attributed to cigarette smoke or oxidative stress.[69] Targeting oxidative/carbonyl stress with pharmacologic antioxidants or boosting the endogenous levels of antioxidants may, therefore, prove to be beneficial in the treatment and management of COPD (Fig 2). To date, however, no clinical studies have shown that antioxidant treatment alone is beneficial or able to lead to the restoration in corticosteroid function. However, compounds such as theophylline have shown a clinically significant effect in enhancing corticosteroid efficacy in patients with COPD.[70] Interestingly, the target-binding profile of theophylline is redox-sensitive and is greater under conditions of oxidative stress, which may account for its efficacy in enhancing steroid efficacy in COPD.[71]

The largest trial of an antioxidant in COPD was the BRONCUS (Bronchitis Randomized on NAC Cost-Utility Study) study which failed to show any overall effect of oral *N*-acetyl cysteine on slowing

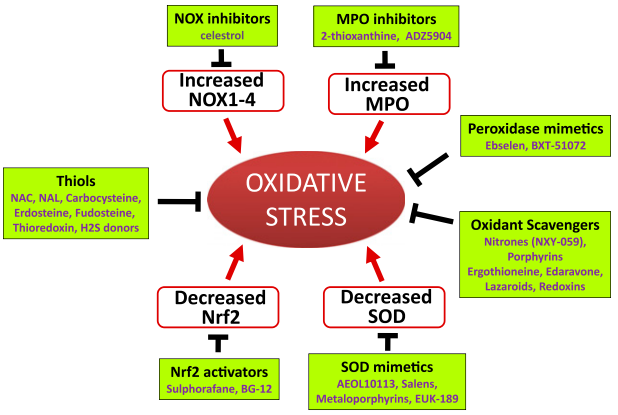

Figure 2. Summary of the different therapeutic approaches to neutralize oxidative stress in COPD. Thiols, oxidant scavengers, and peroxidase mimetics target and neutralize the oxidative stress directly. SOD mimetics and Nrf2 activators attempt to replenish the reduced SOD and Nrf2 activity that is absent in COPD. NOX and MPO inhibitors will neutralize and so reduce oxidative stress. H2S = hydrogen sulfide; MPO = myeloperoxidase; NAC = N-acetylcysteine; NAL = N-acystelyn; NOX = NADPH oxidase; Nrf2 = nuclear erythroid-2-related factor 2; SOD = superoxide dismutase.

disease progression or exacerbation frequency, although there was apparent benefit in the patients not treated with inhaled corticosteroids.[72] An earlier clinical study (Equalife) using a different antioxidant (erdosteine) showed similar findings.[73] The failure of these clinical studies may be attributable to several reasons: the failure of the antioxidant to be targeted to the correct cellular subcompartment where the antioxidant is needed most, the potency of the antioxidant, or the dosage and frequency used in the clinical trials may not have been high enough. Consequently, the development of novel wide spectrum small-molecule antioxidants with good bioavailability and potency are needed for clinical use in COPD. A number of alternative antioxidant strategies (reviewed elsewhere) have been proposed, some of which have shown promise.[74] Perhaps the most encouraging approaches to antioxidant therapy lie with the use of new Nrf2 activators which are significantly more potent than sulforophane[75] and may also prevent oxidative stress-induced autoimmunity.[76] The Nrf2 activator BG-12 successfully completed phase 3 trials for use in multiple sclerosis and is now awaiting approval. However, another Nrf2 activator bardoxolone methyl (CDDO) failed to complete phase 3 due to an excess of serious adverse events. Although both drugs are covalent activators of Nrf2, they differ in the profile of Nrf2-inducible genes that are activated and structurally different and consequently may have different off-target binding profiles to account for the different clinical outcomes. Other promising approaches include the SOD mimetics such as AEOL10113, NOX inhibitors such as celestrol,[77] and MPO inhibitors such as 2-thioxanthine and ADZ5904.[78]

Conclusions

Elevated levels of ROS and carbonyls are found in COPD and these may be associated with increased inflammation, airway remodeling, autoimmunity, and corticosteroid resistance. In addition, systemic oxidative stress may also be a causal link in many COPD comorbidities such as cardiovascular diseases and metabolic syndrome. Local oxidative stress may also promote the development of lung cancer. Following the initial environmental exposure to ROS, the subsequent intracellular sources and chronicity of oxidative stress may be important to understanding the pathophysiology of this disease. The failure of existing antioxidants in COPD studies indicates the need to develop novel more potent antioxidants targeted to the correct intracellular compartment. Combinations of antioxidants, targeting different cellular compartments, may prove more effective than monotherapy. In a similar manner, combining antioxidants with antiinflammatory drugs, bronchodilators, antibiotics, and statins may complement, or in the case of corticosteroids, improve/restore their efficacy.

Acknowledgments

Financial/nonfinancial disclosures: The authors have reported to *CHEST* the following conflicts of interest: Dr Kirkham has received research funding from both industry and research councils over the last 3 years, has been a consultant for the pharmaceutical industry, and has a patent application pending. Research funding sources include Novartis AG; Pfizer, Inc; The Royal Society; Medical Research Council; British Lung Foundation; and the EPSRC. Prof Barnes has received research funding from the Medical Research Council, Wellcome Trust, and the medical charities, and has also received research funding from, and been on scientific advisory boards for, AstraZeneca; Boehringer Ingelheim GmbH; Chiesi Farmaceutici; Cipla; GlaxoSmithKline plc; Novartis AG; Pfizer, Inc; RespiVert Ltd; Teva Pharmaceutical Industries Ltd; and UCB Celltech, all of which are involved in marketing treatments for patients with COPD.

References

1. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med*. 2009; 360(23):2445-2454.
2. Salvi S, Barnes PJ. Is exposure to biomass smoke the biggest risk factor for COPD globally? *Chest*. 2010;138(1):3-6.
3. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med*. 2004;350(26):2645-2653.
4. Hogg JC. Why does airway inflammation persist after the smoking stops? *Thorax*. 2006;61(2):96-97.
5. Taraseviciene-Stewart L, Douglas IS, Nana-Sinkam PS, et al. Is alveolar destruction and emphysema in chronic obstructive pulmonary disease an immune disease? *Proc Am Thorac Soc*. 2006;3(8):687-690.
6. Schaberg T, Klein U, Rau M, Eller J, Lode H. Subpopulations of alveolar macrophages in smokers and nonsmokers: relation to the expression of CD11/CD18 molecules and superoxide anion production. *Am J Respir Crit Care Med*. 1995;151(5): 1551-1558.
7. Ichinose M, Sugiura H, Yamagata S, Koarai A, Shirato K. Increase in reactive nitrogen species production in chronic obstructive pulmonary disease airways. *Am J Respir Crit Care Med*. 2000;162(2 pt 1):701-706.
8. Rahman I, van Schadewijk AA, Crowther AJ, et al. 4-Hydroxy-2-nonenal, a specific lipid peroxidation product, is elevated in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2002;166(4):490-495.
9. Kluchová Z, Petrásová D, Joppa P, Dorková Z, Tkácová R. The association between oxidative stress and obstructive lung impairment in patients with COPD. *Physiol Res*. 2007;56(1): 51-56.
10. Drost EM, Skwarski KM, Sauleda J, et al. Oxidative stress and airway inflammation in severe exacerbations of COPD. *Thorax*. 2005;60(4):293-300.
11. Montuschi P. Exhaled breath condensate analysis in patients with COPD. *Clin Chim Acta*. 2005;356(1-2):22-34.
12. Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. *Am J Respir Crit Care Med*. 2002; 166(12 pt 2):S31-S37.
13. Paredi P, Kharitonov SA, Leak D, Ward S, Cramer D, Barnes PJ. Exhaled ethane, a marker of lipid peroxidation, is elevated in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2000;162(2 pt 1):369-373.
14. Bartoli ML, Novelli F, Costa F, et al. Malondialdehyde in exhaled breath condensate as a marker of oxidative stress in different pulmonary diseases. *Mediators Inflamm*. 2011;2011:891752.
15. Barreiro E, Peinado VI, Galdiz JB, et al; ENIGMA in COPD Project. Cigarette smoke-induced oxidative stress: a role in chronic obstructive pulmonary disease skeletal muscle dysfunction. *Am J Respir Crit Care Med*. 2010;182(4):477-488.
16. Kodgule R, Salvi S. Exposure to biomass smoke as a cause for airway disease in women and children. *Curr Opin Allergy Clin Immunol*. 2012;12(1):82-90.
17. Louhelainen N, Rytilä P, Haahtela T, Kinnula VL, Djukanovi R. Persistence of oxidant and protease burden in the airways after smoking cessation. *BMC Pulm Med*. 2009;9:25.
18. van der Toorn M, Rezayat D, Kauffman HF, et al. Lipid-soluble components in cigarette smoke induce mitochondrial production of reactive oxygen species in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol*. 2009;297(1):L109-L114.
19. Aguilera-Aguirre L, Bacsi A, Saavedra-Molina A, Kurosky A, Sur S, Boldogh I. Mitochondrial dysfunction increases allergic airway inflammation. *J Immunol*. 2009;183(8):5379-5387.
20. Pinamonti S, Leis M, Barbieri A, et al. Detection of xanthine oxidase activity products by EPR and HPLC in bronchoalveolar lavage fluid from patients with chronic obstructive pulmonary disease. *Free Radic Biol Med*. 1998;25(7):771-779.
21. Aaron SD, Angel JB, Lunau M, et al. Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2001;163(2):349-355.
22. Ghio AJ, Pritchard RJ, Dittrich KL, Samet JM. Non-heme (Fe3+) in the lung increases with age in both humans and rats. *J Lab Clin Med*. 1997;129(1):53-61.
23. Janssen-Heininger YM, Persinger RL, Korn SH, et al. Reactive nitrogen species and cell signaling: implications for death or survival of lung epithelium. *Am J Respir Crit Care Med*. 2002;166(12 pt 2):S9-S16.
24. Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A. Protein carbonylation in human diseases. *Trends Mol Med*. 2003;9(4):169-176.
25. Negre-Salvayre A, Coatrieux C, Ingueneau C, Salvayre R. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br J Pharmacol*. 2008;153(1): 6-20.

26. Kirkham PA, Caramori G, Casolari P, et al. Oxidative stress-induced antibodies to carbonyl-modified protein correlate with severity of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2011;184(7):796-802.
27. Kirkham P. Oxidative stress and macrophage function: a failure to resolve the inflammatory response. *Biochem Soc Trans*. 2007;35(pt 2):284-287.
28. Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. *Antioxid Redox Signal*. 2005;7(1-2):42-59.
29. Tsiligianni IG, van der Molen T. A systematic review of the role of vitamin insufficiencies and supplementation in COPD. *Respir Res*. 2010;11:171.
30. Agler AH, Kurth T, Gaziano JM, Buring JE, Cassano PA. Randomised vitamin E supplementation and risk of chronic lung disease in the Women's Health Study. *Thorax*. 2011;66(4):320-325.
31. de Batlle J, Barreiro E, Romieu I, et al. Dietary modulation of oxidative stress in chronic obstructive pulmonary disease patients. *Free Radic Res*. 2010;44(11):1296-1303.
32. Shi MM, Iwamoto T, Forman HJ. Gamma-glutamylcysteine synthetase and GSH increase in quinone-induced oxidative stress in BPAEC. *Am J Physiol*. 1994;267(4 pt 1):L414-L421.
33. Harju T, Kaarteenaho-Wiik R, Soini Y, Sormunen R, Kinnula VL. Diminished immunoreactivity of gamma-glutamylcysteine synthetase in the airways of smokers' lung. *Am J Respir Crit Care Med*. 2002;166(5):754-759.
34. Tomaki M, Sugiura H, Koarai A, et al. Decreased expression of antioxidant enzymes and increased expression of chemokines in COPD lung. *Pulm Pharmacol Ther*. 2007;20(5):596-605.
35. Cheng SL, Yu CJ, Chen CJ, Yang PC. Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COPD. *Eur Respir J*. 2004;23(6):818-824.
36. DeMeo DL, Hersh CP, Hoffman EA, et al. Genetic determinants of emphysema distribution in the national emphysema treatment trial. *Am J Respir Crit Care Med*. 2007;176(1):42-48.
37. Michaeloudes C, Sukkar MB, Khorasani NM, Bhavsar PK, Chung KF. TGF-β regulates Nox4, MnSOD and catalase expression, and IL-6 release in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol*. 2011;300(2):L295-L304.
38. Hwang JW, Rajendrasozhan S, Yao H, et al. FOXO3 deficiency leads to increased susceptibility to cigarette smoke-induced inflammation, airspace enlargement, and chronic obstructive pulmonary disease. *J Immunol*. 2011;187(2):987-998.
39. Pietras T, Szemraj J, Witusik A, et al. The sequence polymorphism of MnSOD gene in subjects with respiratory insufficiency in COPD. *Med Sci Monit*. 2010;16(9):CR427-CR432.
40. Dahl M, Bowler RP, Juul K, Crapo JD, Levy S, Nordestgaard BG. Superoxide dismutase 3 polymorphism associated with reduced lung function in two large populations. *Am J Respir Crit Care Med*. 2008;178(9):906-912.
41. Young RP, Hopkins R, Black PN, et al. Functional variants of antioxidant genes in smokers with COPD and in those with normal lung function. *Thorax*. 2006;61(5):394-399.
42. Kobayashi M, Yamamoto M. Nrf2-Keap1 regulation of cellular defense mechanisms against electrophiles and reactive oxygen species. *Adv Enzyme Regul*. 2006;46:113-140.
43. Mercado N, Thimmulappa R, Thomas CM, et al. Decreased histone deacetylase 2 impairs Nrf2 activation by oxidative stress. *Biochem Biophys Res Commun*. 2011;406(2):292-298.
44. Barnes PJ. Defective antioxidant gene regulation in COPD: a case for broccoli. *Am J Respir Crit Care Med*. 2008;178(6):552-554.
45. Di Stefano A, Caramori G, Oates T, et al. Increased expression of nuclear factor-kappaB in bronchial biopsies from smokers and patients with COPD. *Eur Respir J*. 2002;20(3):556-563.
46. Park HS, Kim SR, Lee YC. Impact of oxidative stress on lung diseases. *Respirology*. 2009;14(1):27-38.
47. Groeger AL, Freeman BA. Signaling actions of electrophiles: anti-inflammatory therapeutic candidates. *Mol Interv*. 2010;10(1):39-50.
48. Donnelly LE, Barnes PJ. Defective phagocytosis in airways disease. *Chest*. 2012;141(4):1055-1062.
49. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. *Chest*. 2006;129(6):1673-1682.
50. Richens TR, Linderman DJ, Horstmann SA, et al. Cigarette smoke impairs clearance of apoptotic cells through oxidant-dependent activation of RhoA. *Am J Respir Crit Care Med*. 2009;179(11):1011-1021.
51. Kirkham PA, Spooner G, Rahman I, Rossi AG. Macrophage phagocytosis of apoptotic neutrophils is compromised by matrix proteins modified by cigarette smoke and lipid peroxidation products. *Biochem Biophys Res Commun*. 2004;318(1):32-37.
52. Bozinovski S, Vlahos R, Zhang Y, et al. Carbonylation caused by cigarette smoke extract is associated with defective macrophage immunity. *Am J Respir Cell Mol Biol*. 2011;45(2):229-236.
53. Barnes PJ, Adcock IM, Ito K. Histone acetylation and deacetylation: importance in inflammatory lung diseases. *Eur Respir J*. 2005;25(3):552-563.
54. Meja KK, Rajendrasozhan S, Adenuga D, et al. Curcumin restores corticosteroid function in monocytes exposed to oxidants by maintaining HDAC2. *Am J Respir Cell Mol Biol*. 2008;39(3):312-323.
55. Ito K, Hanazawa T, Tomita K, Barnes PJ, Adcock IM. Oxidative stress reduces histone deacetylase 2 activity and enhances IL-8 gene expression: role of tyrosine nitration. *Biochem Biophys Res Commun*. 2004;315(1):240-245.
56. Ito K, Ito M, Elliott WM, et al. Decreased histone deacetylase activity in chronic obstructive pulmonary disease. *N Engl J Med*. 2005;352(19):1967-1976.
57. To Y, Ito K, Kizawa Y, et al. Targeting phosphoinositide-3-kinase-delta with theophylline reverses corticosteroid insensitivity in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2010;182(7):897-904.
58. Nakamaru Y, Vuppusetty C, Wada H, et al. A protein deacetylase SIRT1 is a negative regulator of metalloproteinase-9. *FASEB J*. 2009;23(9):2810-2819.
59. Ito K, Barnes PJ. COPD as a disease of accelerated lung aging. *Chest*. 2009;135(1):173-180.
60. Kheradmand F, Shan M, Xu C, Corry DB. Autoimmunity in chronic obstructive pulmonary disease: clinical and experimental evidence. *Expert Rev Clin Immunol*. 2012;8(3):285-292.
61. Kurien BT, Scofield RH. Autoimmunity and oxidatively modified autoantigens. *Autoimmun Rev*. 2008;7(7):567-573.
62. Kirkham PA, Spooner G, Ffoulkes-Jones C, Calvez R. Cigarette smoke triggers macrophage adhesion and activation: role of lipid peroxidation products and scavenger receptor. *Free Radic Biol Med*. 2003;35(7):697-710.
63. Allison ME, Fearon DT. Enhanced immunogenicity of aldehyde-bearing antigens: a possible link between innate and adaptive immunity. *Eur J Immunol*. 2000;30(10):2881-2887.
64. Demedts IK, Bracke KR, Van Pottelberge G, et al. Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;175(10):998-1005.

65. Feghali-Bostwick CA, Gadgil AS, Otterbein LE, et al. Autoantibodies in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2008;177(2):156-163.
66. Di Stefano A, Caramori G, Gnemmi I, et al. T helper type 17-related cytokine expression is increased in the bronchial mucosa of stable chronic obstructive pulmonary disease patients. *Clin Exp Immunol*. 2009;157(2):316-324.
67. Imaoka H, Hoshino T, Takei S, et al. Interleukin-18 production and pulmonary function in COPD. *Eur Respir J*. 2008;31(2):287-297.
68. Kang MJ, Homer RJ, Gallo A, et al. IL-18 is induced and IL-18 receptor alpha plays a critical role in the pathogenesis of cigarette smoke-induced pulmonary emphysema and inflammation. *J Immunol*. 2007;178(3):1948-1959.
69. Culpitt SV, Rogers DF, Shah P, et al. Impaired inhibition by dexamethasone of cytokine release by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2003;167(1):24-31.
70. Ford PA, Durham AL, Russell RE, Gordon F, Adcock IM, Barnes PJ. Treatment effects of low-dose theophylline combined with an inhaled corticosteroid in COPD. *Chest*. 2010;137(6):1338-1344.
71. Marwick JA, Wallis G, Meja K, et al. Oxidative stress modulates theophylline effects on steroid responsiveness. *Biochem Biophys Res Commun*. 2008;377(3):797-802.
72. Decramer M, Rutten-van Mölken M, Dekhuijzen PN, et al. Effects of N-acetylcysteine on outcomes in chronic obstructive pulmonary disease (Bronchitis Randomized on NAC Cost-Utility Study, BRONCUS): a randomised placebo-controlled trial. *Lancet*. 2005;365(9470):1552-1560.
73. Moretti M, Bottrighi P, Dallari R, et al; EQUALIFE Study Group. The effect of long-term treatment with erdosteine on chronic obstructive pulmonary disease: the EQUALIFE Study. *Drugs Exp Clin Res*. 2004;30(4):143-152.
74. Rahman I, MacNee W. Antioxidant pharmacological therapies for COPD. *Curr Opin Pharmacol*. 2012;12(3):256-265.
75. Ichikawa T, Li J, Meyer CJ, Janicki JS, Hannink M, Cui T. Dihydro-CDDO-trifluoroethyl amide (dh404), a novel Nrf2 activator, suppresses oxidative stress in cardiomyocytes. *PLoS ONE*. 2009;4(12):e8391.
76. Pareek TK, Belkadi A, Kesavapany S, et al. Triterpenoid modulation of IL-17 and Nrf-2 expression ameliorates neuroinflammation and promotes remyelination in autoimmune encephalomyelitis. *Sci Rep*. 2011;1:201.
77. Jaquet V, Marcoux J, Forest E, et al. NADPH oxidase (NOX) isoforms are inhibited by celastrol with a dual mode of action. *Br J Pharmacol*. 2011;164(2b):507-520.
78. Churg A, Marshall CV, Sin DD, et al. Late intervention with a myeloperoxidase inhibitor stops progression of experimental chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;185(1):34-43.