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Review

Oxidative stress and lung inflammation in airways disease

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Abstract

Oxidative stress results from an oxidant/antioxidant imbalance in favour of oxidants. A large number of studies have demonstrated that increased oxidative burden occurs in airways diseases, shown by increased marks of oxidative stress in the airspaces and systemically in these patients. There is now substantial evidence that oxidative stress plays an important role in the injurious and inflammatory responses in airways diseases such as asthma and chronic obstructive pulmonary disease (COPD). In addition to these proinflammatory mechanisms resulting from oxidative stress, protective mechanisms such as the upregulation of protective antioxidant genes also occur. At present, effective antioxidant therapy that has good bioavailability and potency is not available. Such drugs are being developed and should in the future allow the hypothesis that oxidative stress is a fundamental factor in the inflammation, which occurs in these airways diseases to be tested.

Keywords: Oxidant; Antioxidant; Oxidative stress; Asthma; Chronic obstructive pulmonary disease

1. Inflammation in airways disease

Asthma and chronic obstructive pulmonary disease (COPD) are conditions associated with airspace inflammation (Jeffery, 2000). The components of the inflammatory response and the site of the inflammation differ in both conditions.

Asthma is a condition of inflammation predominantly in the large airways although the chronic condition is also associated with inflammation in small airways or bronchiolitis (Bousquet et al., 2000).

Chronic obstructive pulmonary disease is a more heterogeneous disease consisting of morphological changes in three regions of the lungs. In the central airways (Saetta et al., 2001), there is chronic inflammation—chronic bronchitis consisting of an inflammatory exudative fluid and cellular infiltrates in the epithelium lining the central airways and the associated glands. The predominant cells in this inflammatory exudate are macrophages and CD8 positive T-lymphocytes. The chronic inflammation in the central airways is also associated with increased number of epithelial, goblet and squamous cells, dysfunction, damage and loss of cilia and enlarged submucosal mucous secreting glands, which results in the mucous hypersecretion that is characteristic of chronic bronchitis.

In peripheral airways, which include small bronchi and bronchioles which have an internal diameter of less than 2 ml, there are inflammatory changes similar to those which occur in the central airways, with exudative cells and fluid in the airway wall and lumen, and goblet and squamous cell metaplasia of the epithelium. The chronic inflammation, which occurs in chronic obstructive pulmonary disease, can initiate a repair process, which produces narrowing of the peripheral airways as a result of scar tissue formation. The repair process in the peripheral airways is characterized by accumulation of fibroblasts and myofibroblasts and extracellular connective tissue matrix. Mediators that drive the accumulation of these cells are incompletely defined but it is likely that several mediators including TGFβ, endothelin-1, insulin-like growth factor, fibronectin and platelet-derived growth factor are involved (Rennard, 1999).

In the lung parenchyma, which includes the gas exchanging surface of the lungs (respiratory bronchioles and alveoli, pulmonary capillary system), destruction can occur in chronic obstructive pulmonary disease commonly pro-

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Table 1
Characteristics of inflammation in chronic obstructive pulmonary disease and asthma

	COPD	Asthma
Cells	• Neutrophils	• Eosinophils
	• Large increase in macrophages	• Small increase in macrophages
	• Increase in $CD8^+$ T lymphocytes	• Increase in $CD4^+$ Th2 lymphocytes
		• Activation of mast cells
Mediators	• Leukotrine B4	• Leukotrine B4
	• Interleukin-8	• Interleukin-, Interleukin-5
	• TNF-α	• (Plus many others)
Consequences	• Squamous metaplasia of epithelium	• Fragile epithelium
	• Parenchymal destruction	• Thickening of basement membrane
	• Mucus metaplasia	• Mucus metaplasia
	• Glandular enlargement	• Glandular enlargement
Response to treatment	• Glucocorticosteroids have little or no effect	• Glucocorticosteroids inhibit inflammation

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ducing the centrilobular form of emphysema, which involves dilatation and destruction of the respiratory bronchioles and surrounding alveoli. Pathological changes occur in large and small airways and in the lung parenchyma to varying degrees in chronic obstructive pulmonary disease patients, which contributes to the heterogeneity of the clinical picture in patients with chronic obstructive pulmonary disease.

In both asthma and chronic obstructive pulmonary disease, chronic inflammation may occur in both large and small airways. However, the inflammation and destruction of the parenchyma in emphysema is exclusively present in chronic obstructive pulmonary disease. An increasing number of biopsy studies of large airways have shown that the inflammatory response in chronic obstructive pulmonary disease and asthma is different. In asthma eosinophils and CDTH-1 type lymphocytes (CD4 positive) using TH1 cytokines predominate whereas in chronic obstructive pulmonary disease CDTH2 lymphocytes (CD8 positive) neutrophils predominate. Macrophages are increased in both conditions (Table 1).

2. Oxidative stress

Reactive oxygen species such as superoxide anion ($O_2^{\cdot -}$) and the hydroxyl radical ($^{\cdot}OH$) are unstable molecules with unpaired electrons, capable of initiating oxidation. Biological systems are continuously exposed to oxidants which can be either generated endogenously by metabolic reactions (e.g. from mitochondrial electron transport during respiration or during activation of phagocytes) or exogenously (such as inhaled from air pollutants or cigarette smoke). To protect against exposure to oxidants, the lungs have a well-developed antioxidant system (Rahman and MacNee, 1996). When an imbalance occurs between oxidants and antioxidants, in favour of oxidants, oxidative stress is said to occur. Increased oxidative stress has been directly linked to oxidation of proteins DNA and lipids, which may cause direct lung injury or induce a variety of cellular responses through the generation of secondary metabolic reactive oxygen species (Gutteridge and Halliwell, 2000). Thus oxidative stress may affect remodeling of extracellular matrix, induce mitochondrial respiration, cell proliferation, and effect protective mechanisms in the lungs, such as the surfactant and the antiprotease screens. Effective repair mechanisms and immune modulation may also be targets of oxidative stress. Oxidative stress is also thought to be a central event in inflammatory responses through the activation of transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1), and thus through signal transduction and gene expression of proinflammatory mediators (Rahman and MacNee, 1998). This article reviews the role of oxidative stress and the pathogenesis of asthma in chronic obstructive pulmonary disease and discusses the molecular mechanisms and pathological consequences of increased oxidative stress in these conditions.

2.1. Cell-derived reactive oxygen species

A common feature of lung inflammation in airways diseases is the development of an inflammatory–immune response, characterised by activation of epithelial cells and resident macrophages, and the recruitment and activation of neutrophils, eosinophils, monocytes and lymphocytes. Once recruited in the airspaces, inflammatory cells may become activated and generate reactive oxygen species in response to various stimuli including cytokines. The activation of phagocytic cells such as macrophages, neutrophils and eosinophils generate superoxide anion, which is then rapidly converted under the influence of superoxide dismutase to hydrogen peroxide (H_2O_2) and $^{\cdot}OH$ is formed nonenzymatically in the presence of Fe^{2+} as a secondary reaction. Reactive oxygen species and reactive nitrogen species can also be generated intracellularly from several

sources, such as mitochondrial respiration, the NADPH oxidase system, xanthine/xanthine oxidase (Fig. 1). The major reactive oxygen species generating enzyme is NADPH oxidase, a complex enzyme system that is present in phagocytes and epithelial cells.

Activation of this enzyme involves a complex assembly of various cytosolic and membrane-associated subunits, resulting in the one-electron reduction of oxygen to $O_2^{\cdot-}$ using NADPH as the electron donor. In addition to NADPH oxidase, phagocytes use other enzymes to produce reactive oxygen species, involving the activity of haem peroxidases (myeloperoxidase) or eosinophil peroxidase. Production of these peroxidases result in the formation of the potent oxidant hypochlorous acid (HOCl) and hypobromous acid (HOBr) from H_2O_2 in the presence of chloride (Cl^-) and bromide (Br^-) ions, respectively. The oxidant burden produced by eosinophils is substantial since these cells possess several times greater capacity to generate $O_2^{\cdot-}$ and H_2O_2 than neutrophils, and the content of eosinophil peroxidase in eosinophils is several times higher than that of myeloperoxidase present in neutrophils (Eiserich et al., 1998; MacPherson et al., 2001; Walsh, 1999; Wu et al., 2000).

Several transition metal salts react with H_2O_2 to form $^{\cdot}OH$. Iron is a critical element in many oxidative reactions (Halliwell and Gutteridge, 1990). Free iron in the ferrous form catalyses the Fenton reaction and the superoxide-driven Haber–Weiss reaction, which generate the $^{\cdot}OH$, a reactive oxygen species, which damages tissues, particularly cell membranes by lipid peroxidation (Gutteridge, 1995). Myeloperoxidase- and eosinophil peroxidase-derived reactive oxygen species can also interact with nitrite (NO_2^-) and H_2O_2 leading to the formation of reactive nitrogen species (RNS). Epithelial cells in the lungs can also release reactive oxygen species (Rochelle et al., 1998). Reactive oxygen species interact with a variety of molecules and donate electrons in biological systems. Reactive oxygen species and reactive nitrogen species also act on certain amino acids such as methionine, tyrosine and cysteine in proteins (e.g. enzymes), which profoundly affect the function of these proteins in inflammatory lung diseases (Halliwell and Gutteridge, 1990).

2.2. Inhaled oxidants and cigarette smoke

Inhalation of cigarette smoke and airborne pollutants, either oxidant gases [such as ozone (O_3), nitrogen dioxide (NO_2), sulphur dioxide (SO_2)] or particulate air pollution results in direct lung damage as well as in the activation of inflammatory responses in the lungs. Cigarette smoke is a complex mixture of over 4700 chemical compounds, including high concentrations of oxidants (10^{14}/oxidant radicals/puff) (Church and Pryor, 1985). The gas phase of cigarette smoke largely contains short-lived oxidants such as $O_2^{\cdot-}$ and nitric oxide (NO). NO and $O_2^{\cdot-}$ react together to form the highly reactive peroxynitrite ($ONOO^-$) molecule (Pryor and Stone, 1993). The tar phase of cigarette smoke contains longer lived radicals such as the semiquinone radicals, which can react with $O_2^{\cdot-}$ to form $^{\cdot}OH$ and H_2O_2 (Nakayama et al., 1989; Zang et al., 1995). Indeed, the aqueous phase of cigarette smoke condensate in epithelial lining fluid may undergo redox recycling for a considerable period of time in smokers (Nakayama et al., 1989; Zang et al., 1995). The tar phase is also an effective metal chelator and can bind iron to produce tar-semiquinone + tar-Fe^{2+}, which can generate H_2O_2 continuously (Nakayama et al., 1989; Zang et al., 1995). Since both cigarette tar and lung epithelial lining fluid contain metal ions, such as iron, Fenton chemistry will result in the production of the $^{\cdot}OH$ which is a highly reactive and potent reactive oxygen species.

2.3. Lipid peroxidation

Reactive oxygen species are highly reactive and when generated close to cell membranes oxidise membrane phospholipids by a process of lipid peroxidation, which may continue as a chain reaction (Fig. 2). Thus, a single $^{\cdot}OH$ can produce many molecules of lipid peroxides in the cell membrane (Gutteridge, 1995). The peroxidation of

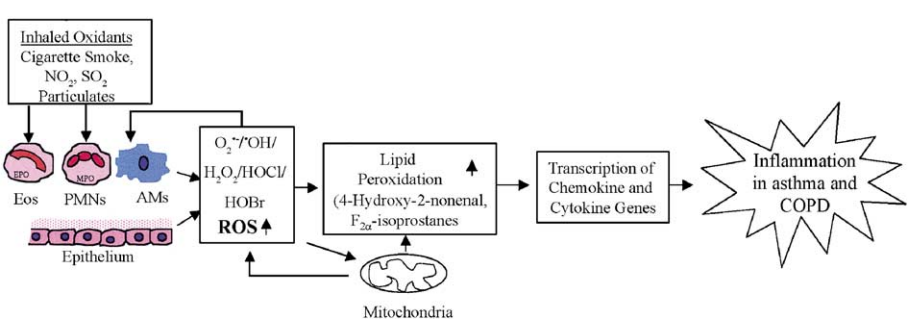

Fig. 1. Mechanisms of reactive oxygen species-mediated lung inflammation in asthma and chronic obstructive pulmonary disease. The inflammatory response is mediated by oxidants either inhaled and/or released by the activated neutrophils, alveolar macrophages, eosinophils and epithelial cells leading to production of reactive oxygen species and membrane lipid peroxidation. This leads to activation of transcription of proinflammatory cytokine and chemokine genes, upregulation of adhesion molecules and increased release of proinflammatory mediators which mediate inflammatory responses in patients with asthma and chronic obstructive pulmonary disease.

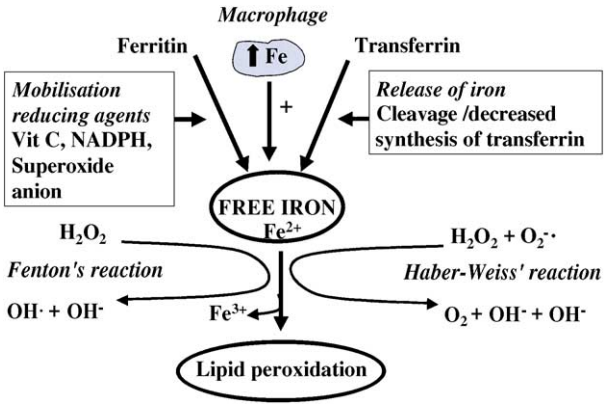

Fig. 2. Free iron catalyse peroxidation membrane lipid peroxidation via Fenton and Haber–Weiss reactions.

polyunsaturated fatty acids impairs membrane function, inactivates membrane-bound receptors and enzymes and increases tissue permeability, processes which have been implicated in the pathogenesis of many forms of lung injury. There is increasing evidence that aldehydes, generated during the process of lipid peroxidation, are also involved in many of the pathophysiological effects associated with oxidative stress in cells and tissues (Gutteridge, 1995). In contrast to free radicals, lipid peroxidation aldehydes are generally stable, can diffuse within, or even escape from the cell and attack targets far from the site of the original free radical event. In addition to their cytotoxic properties, lipid peroxides are increasingly recognised as being important in signal transduction for a number of events in the inflammatory response (Uchida et al., 1999).

An example of this is lipid mediator 4-hydroxy-2-nonenal which is a highly reactive and diffusible end product of lipid peroxidation and is known to induce various cellular events, such as proliferation, apoptosis and activation of signalling pathways (Parola et al., 1999; Uchida et al., 1999).

2.4. Signal transduction and gene expression

ROS have been implicated in the activation of transcription factors such as NF-κB and AP-1, and in signal transduction and gene expression involved in cellular physiologic activities (Rahman and MacNee, 1998). Both environmental and inflammatory cell-derived reactive oxygen species can lead to the activation and phosphorylation of the mitogen activated protein kinase (MAPK) family, including extracellular signal regulated kinase (ERK), c-*Jun* N-terminal kinase (JNK), p38 kinase, and phosphoinositol-3 kinase, leading to increased gene transcription (Guyton et al., 1996; Rahman and MacNee, 1998; Sen, 1998). Activation of members of the MAPK family results in transactivation of transcription factors such as c-*Jun* and CREB-binding protein (CBP) and Elk-1 (Adler et al., 1999; Sen, 1998; Thannickal and Fanburg, 2000). This process eventually results in chromatin remodelling and expression of a range of proinflammatory and antioxidant genes involved in several cellular events including apoptosis, proliferation, and cell differentiation.

Redox-sensitive molecular targets usually contain highly conserved cysteine residues, and their oxidation, nitrosation and the formation of disulfide links are crucial events in redox sensitive signalling. It is hypothesized that oxidation of sulfide groups in signalling proteins causes structural modifications, resulting in the exposure of active sites and consequently protein activation. Such molecular targets include transcription factors (NF-κB, AP-1), signalling molecules such as ras/rac or JNK, protein tyrosine phosphatases (Lander et al., 1997). The intracellular redox state is therefore critical to redox-sensitive signalling pathways and intracellular thiol molecules such as glutathione and thioredoxin are of central importance in the regulation such redox signalling pathways, by reducing disulfide bridges or oxidised cysteine residues (Rahman and MacNee, 2000a,b).

3. Oxidative stress and asthma

There is increasing evidence that inflammation, which is characteristic of asthma, results in increased oxidative stress in the airways (Dworski, 2000); particularly the inflammatory and immune cells in the airways (such as macrophages, neutrophils and eosinophils) release increased amounts of reactive oxygen species in asthmatic patients (Calhoun et al., 1992; Kanazawa et al., 1991; Sedgwick et al., 1990; Vachier et al., 1992). The increased release of reactive oxygen species can result in direct oxidative damage to epithelial cells and cell shedding (Hulsmann et al., 1994). Reactive oxygen species may also evoke bronchial hyperreactivity which is characteristic of asthma (Cortijo et al., 1999; Sadeghi-Hashjin et al., 1996). Many of the triggers for asthma attacks including viral infections, air pollutants such as ozone and particulate air pollution may serve as sources of reactive oxygen species, triggering the increase inflammation which produces asthmatic symptoms. Studies in animal models suggest that reactive oxygen species may contribute to airway hyperresponsiveness by increasing vagal tone due to damage to oxidant sensitive β-adrenergic receptors and by decreasing mucociliary clearance (Adam et al., 1999; Owen et al., 1991) so promoting airway inflammation and hyperreactivity.

Much of the evidence for the involvement of reactive oxygen species in the pathogenesis of asthma is indirect, since there are no specific and reliable methods to assess oxidative stress in vivo. There is, however, good evidence for an increased oxidative burden in asthmatics, since neutrophils isolated from peripheral blood of asthmatic patients generate more reactive oxygen species than cells

from normal subjects, and their ability to produce $O_2^{\cdot-}$ correlates with the degree of airway hyperresponsiveness to inhaled methacholine (Seltzer et al., 1986). Peripheral blood eosinophils also produce increased amounts of reactive oxygen species and reactive nitrogen species such as NO spontaneously and after stimulation ex vivo in asthmatics (Sanders et al., 1995; Sedgwick et al., 1990).

Eosinophil numbers both in bronchoalveolar lavage and blood correlate closely with bronchial hyperresponsiveness (Wardlaw et al., 1988). Eosinophils, alveolar macrophages, and neutrophils from asthmatic patients produce more reactive oxygen species than do those from normal subjects (Schauer et al., 1991; Teramoto et al., 1996). Reactive oxygen species cause direct contraction of airway smooth muscle preparations and this effect is enhanced when the epithelium is injured or removed (Hulsmann et al., 1994). Reactive oxygen species also appear to directly stimulate histamine release from mast cells and mucus secretion from airway epithelial cells, characteristic features in asthma (Krishna et al., 1998).

Numerous surrogate markers of oxidative stress have been shown to be elevated in asthmatic subjects (Dworski, 2000). The concentration of H_2O_2 in exhaled air condensate is increased in stable asthmatics, and may result from decreased dismutation of $O_2^{\cdot-}$ since superoxide dismutase activity is reduced in lung cells of patients with asthma (Comhair et al., 2000; Smith et al., 1997).

Eosinophil peroxidase generated by phagocytes may result in oxidative damage to proteins through bromination of tyrosine residues, as shown by the formation of 3-bromotyrosine in bronchoalveolar lavage of patients with asthma (Wu et al., 2000). 3-bromotyrosine is a specific marker of protein modification by reactive brominating species. Eosinophil peroxidase-generated oxidants can interact with reactive nitrogen species present in asthmatics and promote protein nitration (MacPherson et al., 2001).

The levels of NO are elevated in the exhaled air of patients with asthma (Alving et al., 1993; Silkoff et al., 2000), and may contribute to airway oedema and inflammation (Ashutosh, 2000; Silkoff et al., 2000). The recent finding that NO reduces the potency of β-adrenergic signaling pathways may be a deleterious effect of the elevated reactive nitrogen species in asthma (Adam et al., 1999).

A reaction between NO and $O_2^{\cdot-}$ results in the formation of peroxynitrite anions ($ONOO^-$), a highly reactive oxidant species. $ONOO^-$ adds a nitro group to the 3-position adjacent to the hydroxyl group of tyrosine to produce the stable product nitrotyrosine. $ONOO^-$ induces hyperresponsivess in airways of guinea pigs, inhibits pulmonary surfactant, induces membrane lipid peroxidation and tyrosine and MAP kinase activation, and also damages pulmonary epithelial cells (Dohlman et al., 1993; Groves, 1999; Zhang et al., 2000). The levels of 3-nitrotyrosine have been found to be elevated in the exhaled breath of asthmatic patients (Hanazawa et al., 2000) and immunoreactivity for nitrotyrosine has also been shown to be present in airway epithelium of the airways of patients with asthma (Kaminsky et al., 1999).

3.1. Lipid peroxidation products

Measurement of aldehydes in exhaled breath has been proposed as a means to assess lipid peroxidation in vivo. The levels of lipid peroxides such as 8-isoprostane, a prostaglandin analog, which is a member of the F_2-isoprostane class and a product of the peroxidation of arachidonic acid, hydrocarbons such as ethane and pentane, and nonspecific products of lipid peroxidation such as thiobarbituric acid reactive substances are increased in exhaled breath condensate in patients with asthma (Antczak et al., 1997; Montuschi et al., 1999; Olopade et al., 1997; Paredi et al., 2000). In addition, the levels of 8-isoprostane are also increased in bronchoalveolar lavage fluid of patients with asthma (Dworski et al., 1999). Urinary excretion of the 8-isoprostane $F_2\alpha$ is also increased in mild atopic asthmatics following inhaled allergen provocation (Dworski et al., 2001).

There is also evidence of lipid peroxidation products systemically in asthma as shown by increased levels of plasma F_2-isoprostanes, which in asthma is related to disease severity. Levels of thiobarbituric acid reactive substances have also been shown to be elevated in the plasma of asthmatics and show a negative correlation with the FEV_1 (Calabrese et al., 2000; Wood et al., 2000).

4. Oxidative stress in smokers and patients with chronic obstructive pulmonary disease

The major risk factor for chronic obstructive pulmonary disease is cigarette smoking, which is one of the most potent oxidants. Other factors that may exacerbate chronic obstructive pulmonary disease, such as air pollutants, infections, and occupational dusts, also have the potential to produce oxidative stress (Rahman and MacNee, 1996; Repine et al., 1997).

4.1. Oxidant stress in the airspaces

The oxidant burden in the lungs is enhanced in smokers, both directly from oxidants in cigarette smoke and also by the release of reactive oxygen species from macrophages and neutrophils (Rahman and MacNee, 1996). Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce reactive oxygen species and to release a number of mediators, some of which attract neutrophils and other inflammatory cells into the lungs. Neutrophils and macrophages are known to migrate in increased numbers into the lungs of cigarette smokers, compared with nonsmokers and can generate reactive oxygen species via the NADPH oxidase system (Rahman and MacNee, 1996). Moreover, the lungs of smokers with

airway obstruction have more neutrophils than smokers without airway obstruction (Bosken et al., 1992). Circulating neutrophils from cigarette smokers and patients with chronic obstructive pulmonary disease particularly during exacerbations of chronic obstructive pulmonary disease release more $O_2^{\cdot-}$ (Rahman et al., 1996a). Cigarette smoking is associated with increased content of myloperoxidase in neutrophils (Aaron et al., 2001; Fiorini et al., 2000). There is also increased oxidative stress in airspaces in smokers from the increased release of reactive oxygen species such as $O_2^{\cdot-}$ and H_2O_2 from alveolar macrophages (Rahman and MacNee, 1996; Morrison et al., 1999; Nakashima et al., 1987). Exposure to cigarette smoke in vitro has also been shown to increase the oxidative metabolism of alveolar macrophages (Drath et al., 1979). Subpopulations of alveolar macrophages with a higher density appear to be more prevalent in the lungs of smokers and are responsible for the increased superoxide anion production (Drath et al., 1979; Schaberg et al., 1995).

Hydrogen peroxide in exhaled breath is a direct measurement of oxidant burden in the airspaces. Smokers and patients with chronic obstructive pulmonary disease have higher levels of exhaled H_2O_2 than nonsmokers (Dekhuijzen et al., 1996; Nowak et al., 1996, 1998), and levels are even higher during exacerbations of chronic obstructive pulmonary disease (Dekhuijzen et al., 1996). The source of the increased H_2O_2 is unknown but may in part derive from increased release of $O_2^{\cdot-}$ from alveolar macrophages in smokers (Dekhuijzen et al., 1996). The generation of reactive oxygen species in epithelial lining fluid may be further enhanced by the presence of increased amounts of free iron in the airspaces in smokers (Lapenna et al., 1995; Mateos et al., 1998). This is relevant to chronic obstructive pulmonary disease since the intracellular iron content of alveolar macrophages is increased in cigarette smokers and is increased further in those who develop chronic bronchitis, compared with nonsmokers (Thompson et al., 1991). In addition, macrophages obtained from smokers release more free iron in vitro than those from nonsmokers (Wesselius et al., 1994).

Superoxide anion and H_2O_2 can be generated by the xanthine/xanthine oxidase reaction. Xanthine/xanthine oxidase activity has been shown to be increased in cell free bronchoalveolar lavage fluid and plasma from chronic obstructive pulmonary disease patients, compared with normal subjects and this was associated with increased $O_2^{\cdot-}$ and increased lipid peroxide levels (Heunks et al., 1999; Pinamonti et al., 1996, 1998).

The neutrophil appears to be a critical cell in the pathogenesis of chronic obstructive pulmonary disease. Previous epidemiological studies have shown a relationship between circulating neutrophil numbers and the FEV_1 (Chan-Yeung and Dybuncio, 1984; Van Antwerpen et al., 1995). Moreover, a relationship has also been shown between the change in peripheral blood neutrophil count and the change in airflow limitation over time (Chan-Yeung et al., 1988). In addition, a correlation has been shown between $O_2^{\cdot-}$ release by peripheral blood neutrophils and bronchial hyperreactivity in patients with chronic obstructive pulmonary disease (Postma et al., 1988). Other studies have shown a relationship between reactive oxygen species release from peripheral blood neutrophils and measurements of airflow limitation in young cigarette smokers (Richards et al., 1989).

There is a greater increase in release of reactive oxygen species from peripheral blood neutrophils during exacerbations of chronic obstructive pulmonary disease, which returned to normal when the patients were restudied when clinically stable (Muns et al., 1995; Rahman et al., 1996a, 1997). Other studies have shown that circulating neutrophils from smokers who develop chronic obstructive pulmonary disease have increased reactive oxygen species release, compared with smokers who do not develop the disease (Noguera et al., 1998).

Nitric oxide has been used as a marker of airway inflammation and indirectly as a measure of oxidative stress. Nitric oxide levels have been reported to be higher in the exhaled breath of patients with chronic obstructive pulmonary disease, but not to the high levels reported in asthmatics (Corradi et al., 1999; Delen et al., 2000; Maziak et al., 1998), although not all studies have confirmed this result (Rutgers et al., 1999). Smoking, however, decreases NO levels in breath (Robbins et al., 1997) and in addition, the reaction of NO with $O_2^{\cdot-}$ limits the usefulness of this marker in chronic obstructive pulmonary disease, except perhaps to differentiate from asthma.

Cigarette smoking increases the formation of reactive nitrogen species which results in nitration and oxidation of plasma proteins. The levels of nitrated proteins (such as fibrinogen, transferrin, plasminogen and ceruloplasmin) have been shown to be higher in smokers compared to nonsmokers (Pignatelli et al., 2001). It is likely that aldehydes such as acrolein, acetaldehyde and crotonaldehyde that are abundantly present in cigarette smoke may react with protein–SH and $-NH_2$ groups leading to the formation of a protein-bound aldehyde functional group and is capable of converting tyrosine to 3-nitrotyrosine and dityrosine (Petruzzelli et al., 1997). Nitric oxide and ONOO-mediated formation of 3-nitrotyrosine in plasma and epithelial lining fluid are elevated in smokers (Ichinose et al., 2000). Nitration of tyrosine residues or proteins in plasma leads to the production of 3-nitrotyrosine (Van der Vliet et al., 1994). The levels of nitrotyrosine were higher in airway inflammatory cells obtained by induced sputum from patients with chronic obstructive pulmonary disease, compared to those with asthma (Ichinose et al., 2000). The levels of nitrotyrosine were negatively correlated with the FEV_1 as a percent of predicted values. These direct and indirect studies indicate that an increased reactive nitrogen oxide- and reactive oxygen species-mediated protein nitration and lipid peroxidation may play a role in the pathogenesis of chronic obstructive pulmonary disease.

4.2. Lipid peroxidation products

Isoprostanes are reactive oxygen species catalysed isomers of arachidonic acid and are stable lipid peroxidation products, which circulate in plasma and are excreted in the urine (Morrow and Roberts, 1997). The levels of lipid peroxides such as 8-isoprostane and hydrocarbons, such as ethane and pentane are increased in exhaled air condensate in smokers and in patients with chronic obstructive pulmonary disease (Euler et al., 1996; Habib et al., 1995). The increased levels of these markers of lipid peroxidation correlate with the degree of airways obstruction (Montuschi et al., 2000). Urinary levels of isoprostane $F_2\alpha$-3 have also been shown to be elevated in patients with chronic obstructive pulmonary disease compared with control subjects and are even more elevated during exacerbations of chronic obstructive pulmonary disease (Pratico et al., 1998).

Indirect and nonspecific measurements of lipid peroxidation products, such as thiobarbituric acid reactive substances have also been shown to be elevated in breath condensate and in lungs of patients with stable chronic obstructive pulmonary disease (Nowak et al., 1999). In addition, thiobarbituric acid reactive substances have been shown to correlate negatively with the FEV_1 (Tsukagoshi et al., 2000). Oxidative stress, measured as lipid peroxidation products in plasma has also been shown to correlate inversely with the percent predicted FEV_1 in a population study (Britton et al., 1995), suggesting that in patients with chronic obstructive pulmonary disease, lipid peroxidation may play a role in the progression of the disease.

4-Hydroxy-2-nonenal is a highly reactive and specific diffusible end-product of lipid peroxidation. Increased 4-hydroxy-2-nonenal-modified protein levels are present in airway epithelial cells and in endothelial cells in smokers with airway obstruction, compared to subjects without airway obstruction (Rahman et al., 2001). This demonstrates not only the presence of 4-hydroxy-2-nonenal but that 4-hydroxy-2-nonenal has modified proteins in lung cells to a greater extent in patients with chronic obstructive pulmonary disease. The increased level of 4-hydroxy-2-nonenal-adducts in alveolar epithelium, airway endothelium was inversely correlated with FEV_1.

These studies certainly suggest that there is increased lipid peroxidation in the airways in patients with chronic obstructive pulmonary disease, which may have a role in the pathogenesis of the disease.

5. The consequences of oxidative stress in airways diseases

5.1. Increased airspace epithelial permeability

An important early event following exposure to cigarette smoke is an increase in airspace epithelial permeability (Morrison et al., 1999). The injurious effects of cigarette smoke on human alveolar epithelial cell monolayers, can be shown by increased epithelial cell detachment, decreased cell adherence and increased cell lysis (Lannan et al., 1994).

These effects are in part oxidant-mediated since they can be partially prevented by the antioxidant glutathione (GSH) in concentrations (500 μM) which are present in the epithelial lining fluid. Extra- and intracellular glutathione appears to be critical to the maintenance of epithelial integrity following exposure to cigarette smoke. This was shown in studies of increased epithelial permeability of epithelial cell monolayers in vitro, and in rat lungs in vivo following exposure to cigarette smoke condensate, which is associated with profound changes in the homeostasis of glutathione (Li et al., 1994, 1996; Rahman et al., 1996b, 1995). Indeed, depletion of lung GSH alone can induce increased airspace epithelial permeability both in vitro and in vivo (Li et al., 1995, 1996).

5.2. Oxidative stress and neutrophil traffic in the lungs

The size differential between neutrophils (average diameter 7 μM) and pulmonary capillary segments (average diameter 5 μM), causes a proportion of the circulating neutrophils to deform, and thus move slowly, in order to negotiate the smaller capillary segments. In normal subjects, there is a correlation between neutrophil deformability measured in vitro and the subsequent sequestration of these cells in the pulmonary microcirculation following their reinjection—the less deformable the cells, the increased sequestration of these cells occurs in the pulmonary circulation (Selby et al., 1991). The sequestration of neutrophils in the pulmonary capillaries allows time for the neutrophils to interact and adhere to the pulmonary capillary endothelium, which precedes their transmigration across the alveolar capillary membrane to the interstitium and airspaces of the lungs. There is a transient increase in neutrophil sequestration in the lungs during smoking (MacNee and Selby, 1993), which is due to a decrease in the deformability of circulating neutrophils (Drost et al., 1993). In vitro studies show that the decrease in neutrophil deformability induced by cigarette smoke exposure is abolished by antioxidants, such as glutathione, suggesting that this event is oxidant-mediated (Drost et al., 1992). There is also evidence that oxidative stress reaches the circulation during cigarette smoking, which could decrease the deformability of neutrophils through actin polymerisation (Drost et al., 1992).

Inhalation of cigarette smoke has been shown in animal models to increase neutrophil adhesion to the endothelium of both arterioles and venules, which is thought to be mediated by superoxide anion derived from cigarette smoke, since it can inhibited by pretreatment with superoxide dismutase (Lehr et al., 1993). Neutrophils sequestered in the pulmonary circulation of the rabbit following cigarette smoke inhalation also show increased expression

of adhesion molecules, such as CD18 integrins (Klut et al., 1993).

Activation of neutrophils sequestered in the pulmonary microvasculature could also induce the release of reactive oxygen intermediates and proteases within the microenvironment with limited access for free radical scavengers and antiproteases (Brown et al., 1995). Thus destruction of the alveolar wall, such as occurs in emphysema, could result from a proteolytic insult derived from the intravascular space, without the need for the neutrophils to migrate into the airspaces. Cigarette smoke sequestration in the microcirculation allows chemotaxis to occur. Smoke exposure results in increased chemotactic activity or levels of chemotactic factors in the airspaces (Brown et al., 1995).

5.3. Oxidative stress and proteinase / antiproteinase imbalance

The basis of the protease antiprotease theory in the pathogenesis of emphysema is the development of an increased elastase burden in the lungs and a functional 'deficiency' of α-1 antitrypsin due to its inactivation by oxidants. This "functional α_1-1-antitrypsin deficiency" is thought to be due to inactivation of the α_1-1-antitrypsin by oxidation of the methionine residue at its active site by oxidants in cigarette smoke. This is an oversimplification, because other proteinases and other antiproteinases are likely to have a role. Cigarette smoking causes a transient, but nonsignificant fall in the antiprotease activity of bronchoalveolar lavage fluid (Abboud et al., 1985). Studies assessing the oxidative inactivation of α_1-1-antitrypsin in chronic smokers have failed to produce clear support for this hypothesis.

5.4. Oxidative stress and gene expression

5.4.1. Proinflammatory genes

Numerous studies have shown that markers of inflammation, including interleukin-8 and TNF-α, are increased in the sputum of patients with chronic obstructive pulmonary disease (Barnes, 2000). Genes for many inflammatory mediators, such as interleukin-8, TNF-α, are regulated by transcription factors such as nuclear factor kappa B (NF-κB) (Rahman and MacNee, 1998). NF-κB is present in the cytosol in an inactive form linked to its inhibitory protein IκB. Many stimuli, including cytokines and oxidants, activate NF-κB, resulting in ubiquitination cleaving of IκB from NF-κB and the destruction of IκB in the proteozome (Rahman and MacNee, 1998). This critical event in the inflammatory response is redox-sensitive. In vitro studies using both macrophages and alveolar and bronchial epithelial cells have shown that oxidants cause the release of inflammatory mediators such as interleukin-8, interleukin-1, and nitric oxide and that these events are associated with increased expression of the genes for these inflammatory mediators and increased nuclear binding or activation of NF-κB (Antonicelli et al., 2000). Furthermore, stimuli relevant to the development of exacerbations of chronic obstructive pulmonary disease, such as particulate air pollution that have oxidant properties also activates NF-κB in alveolar epithelial cells (Jimenez et al., 2000).

Thiol antioxidants such as *N*-acetylcysteine and Nacystelin, which have potential as therapies in chronic obstructive pulmonary disease, have been shown in in vitro experiments to block the release of these inflammatory mediators from epithelial cells and macrophages, by a mechanism involving increasing intracellular glutathione and decreasing NF-κB activation (Antonicelli et al., 2000). In addition to a relevant model for chronic obstructive pulmonary disease of cigarette smoke inflammation in the guinea pig, the instillation into the lungs of recombinant superoxide dismutase, a potent antioxidant, prevented both the increased neutrophil influx produced by cigarette smoking, the release of interleukin-8 into bronchoalveolar lavage and NF-κB activation in both alveolar macrophages and in lungs (Nishikawa et al., 1999).

5.4.2. Antioxidant genes

An important response to oxidative stress is the upregulation of protective antioxidant genes.

The antioxidant glutathione is concentrated in epithelial lining fluid compared with plasma and appears to have an important protective role, together with its redox enzymes in the airspaces and intracellularly in epithelial cells (Rahman and MacNee, 1999). Human studies have shown that glutathione is elevated in epithelial lining fluid in chronic cigarette smokers, compared with nonsmokers, an increase which does not occur during acute cigarette smoking (Morrison et al., 1999). The effects of acute and chronic cigarette smoking can be mimicked following intratracheal instillation of cigarette smoke condensate or following exposure of airspace epithelial cells to cigarette smoke condensate in vitro where there is an initial decrease in intracellular GSH with a rebound increase after 12–24 h (Rahman et al., 1995, 1996b,c). This effect in vitro was mimicked by a similar change in glutathione in rat lung in vivo following intratracheal instillation of cigarette smoke condensate, associated with an increase in the oxidised form (GSSG) (Rahman et al., 1995). The increase in GSH following cigarette smoke exposure is due to transcriptional upregulation of mRNA for γ-glutamyl cysteine synthetase, the rate-limiting enzyme for glutathione synthesis (Rahman et al., 1996c, 1998). The mechanism of the upregulation of γGCS mRNA is by the activation by cigarette smoke of the redox-sensitive transcription factor activator protein-1 (AP-1) (Rahman et al., 1998, 1999). A proximal AP-1 site in the promoter region of the gene is critical for the regulation of γ-glutamylcysteine synthetase gene expression in response to various oxidants including cigarette smoke (Rahman et al., 1999). These events are likely to account for the increased glutathione levels seen in the epithelial lining fluid in chronic cigarette smokers,

which acts as a protective mechanism, whereas the more injurious effects of cigarette smoke may occur repeatedly during and immediately after cigarette smoking when the lung is depleted of antioxidants, including glutathione. Rats exposed to whole cigarette smoke for up to 14 days show increased expression of a number of other antioxidant genes in bronchial epithelial cells, including manganese superoxide dismutase and metallothionein and glutathione peroxidase (Gilks et al., 1998).

The c-*fos* gene belongs to a family of growth and differentiation-related immediate early genes, the expression of which generally represents the first measurable response to a variety of chemical and physical stimuli. Studies in various cell lines have shown enhanced gene expression of c-*fos* in response to cigarette smoke condensate. These effects of cigarette smoke condensate can be mimicked by peroxynitrite and smoke-related aldehydes in concentrations that are present in cigarette smoke condensate (Muller and Gebel, 1998). The effects of cigarette smoke condensate can be enhanced by pretreatment of the cells with buthionine sulfoxamine to decrease intracellular glutathione and can be prevented by treatment with *N*-acetylcysteine, a thiol antioxidant (Muller and Gebel, 1998). These studies emphasise the importance of intracellular levels of the antioxidant glutathione in the regulation of gene expression (Rahman and MacNee, 1999).

Thus, oxidative stress, including that produced by cigarette smoke, causes increased gene expression of both injurious proinflammatory genes by oxidant-mediated activation of transcription factors such as NF-κB, but also activation of protective genes such as γ-glutamylcysteine synthetase. A balance may therefore exist between pro- and "antiinflammatory" gene expression in response to oxidants, which may be critical to whether cell injury occurs (MacNee and Rahman, 2000).

6. Therapeutic option to redress the oxidant / antioxidant imbalance

Various options are possible to enhance the lung antioxidant screen (MacNee, 2001). One approach would be the molecular manipulation of antioxidant genes, such as glutathione peroxidase or genes involved in the synthesis of glutathione, such as γGCS or by developing molecules with activity similar to those of antioxidants enzymes such as catalase and superoxide dismutase.

Another approach would simply be to administer antioxidant therapy. This has been attempted in cigarette smokers using various antioxidants such as vitamin C and vitamin E (MacNee, 2001). The results have been rather disappointing, although vitamin E has been shown to reduce oxidative stress in patients with chronic obstructive pulmonary disease (Hoshino et al., 1990). Attempts to supplement lung glutathione have been tried using the glutathione or its precursors (Rahman and MacNee, 2000b). The cysteine donating compound *N*-acetylcysteine acts as a cellular precursor of GSH and becomes de-acetylated in the gut to cysteine following oral administration. It reduces disulphide bonds and has the potential to interact directly with oxidants. *N*-acetylcysteine given in doses of 600 mg three times daily for 5 days produced a significant increase in plasma GSH levels, but no associated rise in bronchoalveolar lavage GSH or in lung tissue (Bridgeman et al., 1991, 1994). These data seem to imply that producing a sustained increase in lung GSH is difficult using *N*-acetylcysteine in subjects who are not already depleted of glutathione. In spite of this, a recent metaanalysis of clinical studies using *N*-acetylcysteine has shown positive results, particularly in reducing exacerbation rates in chronic obstructive pulmonary disease, which have at least in part been attributed to the antioxidant properties of this drug (Stey et al., 2000).

The 'proof of principal' for a role of oxidative stress in the pathogenesis of chronic obstructive pulmonary disease and asthma will come from clinical studies on the effectiveness of antioxidant therapy which has good bioavailability and potency.

In summary, there is now very good evidence for the presence of oxidative stress in chronic obstructive pulmonary disease and asthma. Oxidative stress is critical to the inflammatory response in chronic obstructive pulmonary disease and asthma through the upregulation of redox-sensitive transcription factors and hence proinflammatory gene expression, but also in the protective mechanisms in the form of upregulation of antioxidant genes. Inflammation itself produces oxidative stress in the lungs in asthma and chronic obstructive pulmonary disease. Knowledge of the mechanisms of the effects of oxidative stress should in future allow the development of novel antioxidant therapy which will test the hypothesis that oxidative stress is involved in the pathogenesis of chronic obstructive pulmonary disease and asthma, not only by direct injury to cells, but as a fundamental factor in the inflammation in these airways diseases.

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