Nitric Oxide in Pulmonary Processes:
Role in Physiology and Pathophysiology of Lung Disease
ed. by M. G. Belvisi and J. A. Mitchell

CHAPTER 2
Reactive Oxygen and Reactive Nitrogen Species in the Lung

Gregory J. Quinlan and Nicholas J. Lamb

Unit of Critical Care, Royal Brompton Hospital, National Heart and Lung Institute at Imperial College, Sydney Street, London SW3 6NP, UK

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated as contributing to the pathogenesis of a broad spectrum of diseases [1, 2]. Historically, oxygen free radicals were primarily considered to be aggressive species, indeed the superoxide ($O_2^{\cdot-}$) theory of oxygen toxicity is based on this hypothesis, (reviewed in 3). There is circumstantial evidence to support this view, some of which will be reviewed elsewhere in this chapter. However, other roles for free radicals – or more appropriately ROS and RNS – have recently emerged, most notably as signal or second messenger molecules. It seems therefore that these species can have differing effects which are dependent on their levels of production and on antioxidant defences. This chapter will mainly be concerned with the deleterious consequences associated with these reactive species, particularly in the lung, with special reference to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS).

1.1. Definitions

A biological definition of a free radical is "any chemical species capable of independent existence that contains one or more unpaired electrons" [4]. Classically, free radicals are thought of as highly reactive species, but this is often not the case. Ground state molecular oxygen and nitric oxide (NO) have unpaired electrons, and therefore are free radicals, although neither are particularly reactive species. However, other related reactive oxidants like ozone and peroxynitrite ($ONOO^-$), or species such as hydrogen peroxide (H_2O_2), with the potential to form reactive species, are not free radicals. For this reason, the terms reactive oxygen species (ROS) for oxygen containing species, and reactive nitrogen species (RNS) for nitrogen containing species, have been introduced to allow free radicals and other related species to be included within common definitions.

2. Reactive Oxygen Species (ROS)

Oxygen is an essential requirement for aerobic life forms, as the terminal electron acceptor at the end of the respiratory chain. During aerobic metabolism carbohydrate is oxidised whilst oxygen is reduced by the sequential addition of four electrons, leading to the formation of water. Various ROS are produced as intermediates during this process (equations 1–4):

$$O_2 + e^- + H^+ \rightarrow HO_2^{\cdot} \text{ (hydroperoxyl radical)} \quad (1)$$
$$HO_2^{\cdot} \rightarrow H^+ + O_2^{\cdot -}$$

$$O_2^{\cdot -} + 2\,H^+ + e^- \rightarrow H_2O_2 \quad (2)$$

$$H_2O_2 + e^- \rightarrow OH^- + {}^{\cdot}OH \text{ (hydroxyl radical)} \quad (3)$$

$${}^{\cdot}OH + e^- + H^+ \rightarrow H_2O \text{ (water)} \quad (4)$$

$O_2^{\cdot -}$ although a free radical anion, is a weak oxidising agent, capable of oxidising thiols and ascorbic acid. It is, however, a much stronger reducing agent, capable of reducing several iron complexes. At physiological pH it is unstable and rapidly dismutates to H_2O_2, a process which is accelerated by the antioxidant enzyme superoxide dismutase (SOD).

H_2O_2 is an uncharged molecule, readily soluble in water, with the ability to enter and leave cells easily. It is not a very reactive species, but can ultimately lead to the formation of the most aggressive oxygen free radical known, the hydroxyl ($^{\cdot}OH$) radical. H_2O_2 levels are regulated *in vivo* by glutathione peroxidase, and catalase antioxidant enzymes.

The $^{\cdot}OH$ radical can be formed via the iron (Fenton reaction) or copper catalysed decomposition of H_2O_2. This reaction emphasises the importance

of redox active transition metal ions in free radical chemistry and oxygen toxicity. The ·OH radical is an extremely reactive oxidant that attacks most biological molecules at almost diffusion-controlled rates. This extreme reactivity, however, limits its ability to cause damage at any distance from its site of formation, although it can initiate radical chain reactions such as lipid peroxidation [4]. Recently iron-independent mechanisms for *in vivo* ·OH production have been proposed, either via the decomposition of peroxynitrous acid [5] or from the reaction of $O_2^{\cdot-}$ with hypochlorous acid (a neutrophil derived oxidant) [6]. Both mechanisms are, however, still open to debate [7, 8].

Ozone is a powerful oxidant and toxic pollutant which has been implicated in various respiratory disorders including asthma [9]. It is capable of causing oxidative damage to biomolecules such as DNA, lipids, and carbohydrates [10, 11].

Ground state molecular oxygen (O_2) is classified as a free radical as it contains two unpaired electrons with parallel spins. This spin restriction limits its reactivity. It can, however, react by accepting electrons one at a time, in reactions involving transition metal ions such as iron and copper. More reactive forms of oxygen can also be formed, as a result of energy input into ground state oxygen, and are known collectively as singlet oxygen. Two forms exist, ($^1\Sigma g^+O_2$) is the most reactive, and is a free radical containing two unpaired electrons with opposite spins. It rapidly decays to the ($^1\Delta gO_2$) form, which is not a free radical as both electrons now occupy the same orbital. Singlet oxygen can also be formed from the interaction of H_2O_2 with the hypochlorite ion, a reaction that may be of biological significance. Its formation *in vivo* is most often associated with photosensitization reactions.

Hypochlorous acid is a potent bleaching agent, produced by the lysosomal enzyme, myeloperoxidase, of activated neutrophils. Its key function is as a microbial killing agent. Production of this powerful oxidant can, however, also have detrimental effects. It readily oxidises or chlorinates many biological molecules including thiols, amines and nucleotides [12], and causes intramolecular crosslinking of proteins [13]. Hypochlorous acid can interact with other ROS or decompose to form other damaging oxidants including the ·OH radical (either independently [6] or via iron catalysis [8]). Recently hypochlorous acid has been shown to form a potent chlorinating and nitrating species on interaction with nitrite [13, 14].

2.1. Organic Oxygen Radicals

Lipid peroxides can be formed in biological systems by a variety of mechanisms. Purposeful enzyme catalysed lipid peroxidation occurs in both animal and plant tissues to produce bioactive substances collectively known as eicosanoids. Various ROS are also capable of initiating lipid peroxida-

tion that can lead to deleterious consequences. Singlet oxygen is capable of reacting directly with carbon-carbon double bonds to produce lipid hydroperoxides [16]. Other non-radical oxidants such as ozone, $ONOO^-$, and hypochorite have also been implicated in lipid peroxidation processes [17–19]. The $^{\cdot}OH$ radical, if formed locally reacts with unsaturated fatty acids resulting in the formation of peroxy radicals capable of initiating further peroxidation. Stable lipid peroxides can also be formed, these are not free radicals, but in the presence of iron or copper ion catalysts form alkoxyl or peroxyl radicals, which are also able to propagate the peroxidation process.

2.2. Reactive Nitrogen Species (RNS)

NO, contrary to popular belief is not a particularly reactive molecule, except under certain circumstances (reactions with other free radicals). It is an environmental pollutant and is also found in cigarette smoke. NO is produced *in vivo* both constitutively and inducibly via the NO synthase (NOS) enzyme systems. Its biological functions are indistinguishable from those of endothelial-derived relaxing factor (EDRF) and may also function as an antioxidant by inhibiting the ROS producing enzyme xanthine oxidase [20], by scavenging $O_2^{\cdot-}$ [21], and by acting as a chain breaking antioxidant.

Nitrogen dioxide (NO_2) is a free radical gas, a pollutant, and a constituent of cigarette smoke. It is a powerful oxidant and may therefore be of some significance to respiratory diseases such as asthma [22]. NO_2 can be formed by reaction of nitrogen with molecular oxygen. However, this reaction is thought to be of little physiological relevance, as it is out competed by $ONOO^-$ formation [23].

$ONOO^-$ is not a free radical; it is however, a powerful oxidant, formed from the reaction of $O_2^{\cdot-}$ with NO (reviewed in [23]), and possibly by NOS enzymes directly [24]. As an oxidant, $ONOO^-$ can damage lipids, DNA, and proteins [25–27]. It is also a nitrating and nitrosating species, able to nitrate tyrosine and tryptophan residues [28, 29], and nitrosate thiol groups to form nitrosothiols [30, 31]. It has been suggested that the major deleterious effect associated with its formation *in vivo*, is not as an oxidant but rather as a nitrating agent of proteins, the modification of which can result in a loss of function. High and low molecular mass nitrosothiols may act as an *in vivo* sink for NO, indicating a positive role for $ONOO^-$ formation *in vivo*. Indeed, reports have shown the ability of $ONOO^-$ to induce vasorelaxation, some via thiol dependent release of NO [32]. Other reports suggest further beneficial effects may be associated with the scavenging of $O_2^{\cdot-}$, as NO has been shown to protect against this type of ROS-mediated lung injury [33, 34]. Additionally, physiologically relevant doses of $ONOO^-$ have been found to be cardioprotective in a cat model of myocardial ischaemia and reperfusion [35].

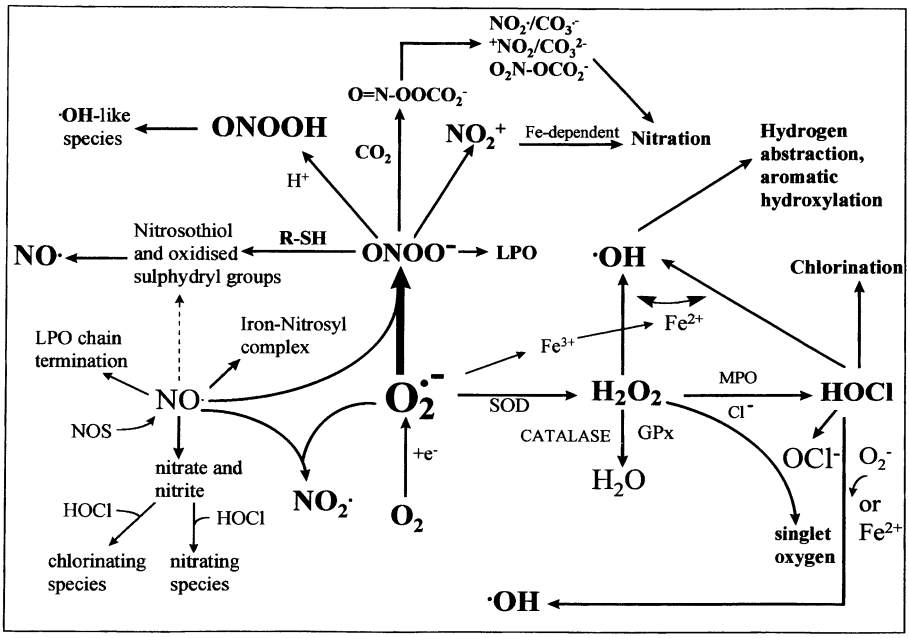

Figure 1. The interactions which may lead to the production of ROS and RNS *in vivo* are depicted. (LPO) lipid peroxidation, (GPx) glutathione peroxidase, (SOD) superoxide dismutase.

2.3. Summary

It is clear then that numerous ROS and RNS can be produced *in vivo*, and that there is a complex interrelationship between these species, which is further influenced by transition metal ion catalysts and antioxidants (see Fig. 1).

3. ROS and RNS and Their Role in Lung Injury

3.1. Acute Respiratory Distress Syndrome (ARDS)

ARDS is an acute form of inflammatory lung injury, precipitated by a variety of predisposing causes, many not directly related to the lung. It is characterised by non-cardiogenic pulmonary oedema and carries with it a high instance of mortality (for reviews see [36, 37]). ROS and RNS have been implicated as contributory factors to the onset and progression of ARDS, such species arise as a result of various processes (see Fig. 2).

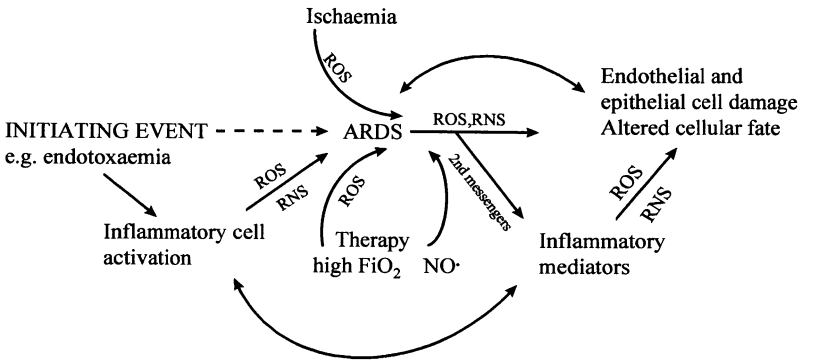

Figure 2. Possible sources of ROS and RNS in ARDS are illustrated.

3.2. Hyperoxia

It is now known that the deleterious effects of oxygen are attributable to the reactive nature of its reductive intermediates, this was first proposed as a theory by Gerschman and colleagues in 1954 [38]. ROS and RNS arise *in vivo* principally as a result of normal cellular metabolic processes. 1% of all oxygen consumed during aerobic respiration leaks from the respiratory chain of mitochondria as $O_2^{\cdot-}$, which is scavenged by endogenous antioxidants. However, exposure to normabaric concentrations of oxygen greater than those found in normal air during hyperoxia, leads to increased leakage of $O_2^{\cdot-}$ from mitochondria and other organelles, with a consequent increase in H_2O_2 (for reviews see [39, 40]). The pathology of oxygen toxicity in the lungs of humans results in tissue damage and can lead to ALI [9]. Oxygen-induced lung damage leads to atelectasis, fibrin deposition, thickening and hyalinisation of alveolar membranes [41], and alterations in the composition and properties of surfactant [42]. Evidence for the involvement of oxidants in this form of lung injury is further strengthened by findings showing protection from oxygen toxicity after previous exposure to hyperoxia [43], endotoxin [44], or cytokines [45]. Protection results from the induction of lung antioxidant defences at the time of primary exposure. These defences include upregulation of antioxidant enzymes (SODs, catalase, glutathione peroxidases), iron-oxidising enzymes (caeruloplasmin) [46, 47], and protective peptides [48]. Recently, inhibitors of anion exchange and L-arginine have been shown to attenuate this form of lung injury, implicating the $O_2^{\cdot-}$ anion and $ONOO^-$ in the injury process [49].

3.3. Ischaemia Reperfusion Injury

When tissues are deprived of oxygen (ischaemia) or oxygen tensions are reduced (hypoxia), biochemical changes result in cell damage and death. If

oxygen is restored to tissues they can survive, but this is dependent on the length of time the tissue was deprived of oxygen and also on the type of tissue. However, studies have shown that on reoxygenation an additional cellular injury occurs which is mediated in part by the production of ROS and is known as ischaemia/reperfusion injury [50]. Several mechanisms for ROS production in ischaemia/reperfusion injury have been proposed but it is now thought that they may be formed as a result of changes to the mitochondrial electron transport chain during ischaemia/hypoxia, which result in increased leakage of $O_2^{\cdot-}$ when tissues are reperfused. The formation of eicosanoids relies on single electron transfer reactions, these biosynthetic processes are upregulated during ischaemia and may lead to ROS formation (reviewed in [51]). Inflammatory cell activation and ROS release during the respiratory burst may also be involved in ischaemia/reperfusion injury, although some literature suggests that this is not a feature in the initial stages of injury [51]. Much research into ischaemia/reperfusion injury has concentrated on the enzyme xanthine oxidase (XOD) and its role in ROS production during ischaemia/reperfusion [50, 52, 53]. The enzyme exists in two isoforms, and is rate limiting in purine catabolism. The oxidase form of XOD is produced by limited proteolysis or oxidative modification [54] as a result of neutrophil activation [55]. XOD catalyses the breakdown of purines to uric acid, coupled with the reduction of oxygen to $O_2^{\cdot-}$ and H_2O_2. Appreciable conversion of the enzyme occurs during ischaemia, and when oxygen is reintroduced, ROS are formed. Additionally, levels of substrates (hypoxanthine and xanthine) for the enzyme become elevated during the ischaemic period due to aberrant ATP metabolism [54], so increasing the prooxidant potential of XOD. Recently, substrate formation rather than enzyme conversion has been shown to be of key importance in myocardial ischaemia/reperfusion injury [56]. A potential for XOD-mediated ROS production in patients with ARDS exists, as plasma and bronchoalveolar lavage fluid (BAL) hypoxanthine levels are found to be significantly elevated in non-surviving patients [57] and XOD is detectable in plasma from such patients [58]. Lung injury in the form of high permeability pulmonary oedema is seen in animal studies where XOD and xanthine are instilled into the lungs of rabbits or rats [59, 60]. Further, lipopolysaccharide (LPS)-induced pulmonary oedema in the mouse lung is associated with the induction of XOD activity [61].

The liver and the gut are particularly rich in XOD [62], which is present in relatively low amounts in the heart and lung [63], casting doubt on the role of XOD in ischaemia/reperfusion injury in these organs. However, recent evidence shows that XOD has a heparin-like binding site and is capable of binding to endothelial cells [64]. So raising the possibility that XOD may be released into the circulation, and may subsequently bind to the endothelium within organs where it is not normally found. Indeed, recent studies in animal models of gut and liver induced surgical ischaemia show lung injury attributable to the activity of XOD [65]. Further, XOD

has now been demonstrated in animal [66], and human endothelial cells [67] where it contributes to lung injury through several mechanisms including oxidant formation [68]. XOD may contribute to ischaemia/reperfusion injury by promoting neutrophil sequestration in the lung by an $O_2^{\cdot-}$-dependent mechanism [69] and by contributing to their adherence to cultured endothelial cells in the presence of xanthine [70]. It may also promote cytokine production and NF-κ B activation in lungs, as seen in a mouse models of haemorrhagic shock [71, 72].

3.4. Inflammatory Cells

Activated neutrophils and macrophages contain a membrane-bound nicotinamide dinucleotide phosphate (NADPH) oxidase enzyme, which produces $O_2^{\cdot-}$ (the respiratory burst), and contributes to bacterial cell killing [73]. Recently, similar enzyme systems have been found in other cell types including lung fibroblasts [74]. Increased levels of $O_2^{\cdot-}$ production have been demonstrated in animal models of ALI induced by oleic acid and endotoxemia [75, 76], and the NADPH oxidase inhibitor apocynin is known to attenuate sepsis induced lung injury in guinea-pigs [77]. Under normal physiological conditions $O_2^{\cdot-}$ rapidly dismutates to H_2O_2 an effect which can also be seen during the respiratory burst of neutrophils [78]. H_2O_2 is detectable in breath condensates of patients with ARDS, at significantly elevated levels compared to ventilated non-ARDS control patients [79], and in patients with hypoxemic respiratory failure [80]. Additionally it can be detected in the urine of critically ill patients with sepsis and ARDS [81], (reviewed in [82]). The $^{\cdot}OH$ radical is formed from H_2O_2 in the presence of redox active iron, and recently this form of iron has been measured in human BAL fluid [83]. This may have implications for ROS mediated lung injury in acute inflammatory states such as ARDS and ALI. Indeed evidence for $^{\cdot}OH$ mediated damage to BAL fluid protein measured as non-enzyme formed tyrosine isomers (makers of $^{\cdot}OH$ formation), has been found in these patients [84]. The $^{\cdot}OH$ radical is also capable of initiating lipid peroxidation. Animal models of acute lung injury show increases in non-specific markers of lipid peroxidation, such as thiobarbituric reactive substances (TBARS) in lung tissue [85] and conjugated dienes in plasma [86], the levels of which are related to the degree of lung injury. In humans with ARDS, elevated plasma levels of TBARS have been found accompanied by decreased levels of unsaturated fatty acids and vitamin E [87]. Plasma TBARS levels have also been shown to correlate well with the Murray lung injury score in ARDS patients, although the mechanisms involved may not be entirely neutrophil dependent [88]. Mechanical ventilation may also contribute to plasma lipid peroxidation in such critically ill patients [89]. However, 4-hydroxy-2-nonenal (HNE) is a more reliable indicator of lipid peroxidation. It is a specific aldehydic n-6 fatty acid oxidation prod-

uct, which has been demonstrated *in vivo* (reviewed in [90]). HNE can be cytotoxic, chemotactic, inhibit some enzymes and be produced by lung neutrophils in the rat [91]. Elevated levels of this bio-active aldehyde have been reported in the plasma of patients with ARDS [92]. HNE and other products of lipid peroxidation are markers of oxidative damage, but additionally may contribute to injurious processes in the lung. For instance, linoleic acid hydroperoxides which induce broncho- and vasoconstriction in isolated rat lungs [93], are toxic to endothelial cells [94], and lead to increased phospholipid oxidation [95]. The other oxidant produced by neutrophils is hypochlorous acid. Evidence to implicate this aggressive ROS in lung injury is strengthened by findings of subcellular matrix damage of the endothelium [96], and loss of lung surfactant surface tension function [97]. Recently, chlorinated tyrosine residues (markers of hypochlorous acid formation) on BAL fluid proteins have been detected in patients with ARDS at significantly elevated levels compared to ventilated and normal control groups, findings suggestive of a role for this oxidant in lung injury seen in these patients [84].

It is now clear that RNS are formed in a variety of inflammatory disease states where NO may be formed in excess. Upregulation of inducible NOS leads to increased formation of NO. Under such conditions, where there are high levels of both NO and $O_2^{\cdot-}$, $ONOO^-$ formation is favoured. This reaction is very fast and 'out competes' SOD enzymes, and occurs at a much faster rate than iron catalysed $^{\cdot}OH$ formation. Promoting some to suggest that $ONOO^-$ is chiefly responsible for the oxidative damage seen in pathological conditions (reviewed in [2]). $ONOO^-$ is a powerful oxidant, but supportive evidence for its formation *in vivo* comes mainly from measurement of products formed due to its action as a nitrating species, in particular its ability to nitrate tyrosine residues [98]. The precise mechanism of the nitration reaction is unclear, but may involve an iron-dependent reaction in which nitronium ions are formed and react with tyrosine [98] or via the formation of a reactive intermediate with carbon dioxide [99, 100]. Macrophages [101], neutrophils [102], and cultured vascular endothelium [103], have all been implicated as sources of $ONOO^-$. In addition nitrotyrosine has been detected by immunohistochemistry in lung slices [104] and by high-pressure liquid chromatography (HPLC) in BAL fluid proteins from patients with ARDS [84].

The mechanisms of $ONOO^-$ mediated lung injury are varied, and may include its ability as an oxidant to cause lipid peroxidation [105], glutathione depletion [106] and other forms of $^{\cdot}OH$-like damage. It can nitrate lung surfactant proteins (SP-A) leading to a decreased ability to aggregate lipids [107] and decreased mannose binding ability [108] resulting in impaired surfactant function. It may also impair sodium transport [109] and surfactant synthesis by alveolar type II cells [110]. All these adverse effects might be exacerbated by the use of inhaled NO therapy, which is sometimes used as a treatment for pulmonary hypertension [111]. Indeed,

numerous studies in animals have demonstrated damage and dysfunction associated with inhaled NO treatment [111].

3.5. Antioxidants

The extracellular iron-binding and iron-oxidising anti-oxidant proteins transferrin and caeruloplasmin, are compromised in patients with ARDS [112, 113] and free redox active iron can be detected in the plasma of some patients [114]. Likewise in BAL fluid of patients with ARDS, abnormalities in transferrin and caeruloplasmin are present [115]. Recently, redox active iron has been demonstrated in normal BAL fluid, and elevated levels of transferrin iron saturation have been found in BAL fluid from patients with ARDS [23]. Deficiencies in these antioxidants may therefore contribute to increased oxidative damage and nitration, via iron catalysed mechanisms, in these patients. Interestingly, plasma levels of the intracellular iron-binding protein ferritin have been shown to be a predictive mortality factor in ARDS [116]. Reduced glutathione contains a thiol group, it reacts with oxidants such as H_2O_2, hypochlorous acid, and $ONOO^-$, and protects proteins from aldehydic modification [117], and is a cofactor for the antioxidant protein glutathione peroxidase. Extracellular levels of glutathione are low except in lung lining fluid, but in patients with ARDS this is not the case as most of the glutathione is oxidised [118], suggestive of increased oxidative stress in the lungs of these patients. In plasma, glutathione levels are very low, but there are other high molecular mass thiol containing proteins (mainly albumin) which perform similar antioxidant functions, levels of which are reduced in patients with ARDS [119]. Other plasma and lipid phase antioxidants such as ascorbic acid and vitamin E are similarly reduced in these patients [120, 121]. To compensate for this deficiency in antioxidant levels treatment regimes involving the use of exogenous antioxidants such as N-acetylcysteine have been employed with limited success [122].

3.6. Other Lung Diseases

ROS and RNS have been implicated in many other respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD), in which iron [123], inflammatory cell activation [124] and ROS [124] production are implicated (for review see [125]). XOD formation may also contribute to oxidative stress in these patients [126]. The underlying mechanism of asthma is at present unclear, but ROS formed by inflammatory cells are implicated [127]. Further, lung cells recovered from asthmatics can generate increased amounts of ROS and have reduced antioxidant (SOD) activity [127], other antioxidants are also reduced in the plasma of

these patients (glutathione peroxidase) [128]. Elevated levels of inducible NOS and NO [129] are also seen, raising the possibility of $ONOO^-$ production in these patients. In addition, inhaled oxidant pollution gases are implicated in asthma (see previous sections), as are particulate air pollution products (PM10s). Recent findings show that these particles exhibit oxidant activity [130].

Patients with cystic fibrosis experience elevated oxidative stress due to chronic lung inflammation, and inadequate absorption of dietary antioxidants. Increased levels of markers of oxidative damage to lipids and DNA are found in such patients [131, 132], which in the case of lipids correlates with pulmonary dysfunction. Markers of RNS formation are elevated in the lungs of patients with idiopathic pulmonary fibrosis implicating such species in the disease process [133]. Paraquat poisoning causes damage to pulmonary tissue via the redox cycling activity of this herbicide which results in the formation ROS capable of damaging DNA [134].

4. ROS and RNS as Second Messengers

NO is a known second messenger, but recently other RNS/ROS have been attributed roles in intracellular signal transduction pathways. Many cellular processes including apoptosis [135], are thought to be regulated by subtoxic levels of ROS/RNS. Indeed the anti-apoptotic gene *bcl-2* has been shown to operate by lowering intracellular ROS production [136, 137]. The level of oxidative stress is critical in the signalling process, low concentrations of H_2O_2 will induce apoptosis, but higher concentrations lead to unwanted cell death via necrosis [138], similar findings are seen with other

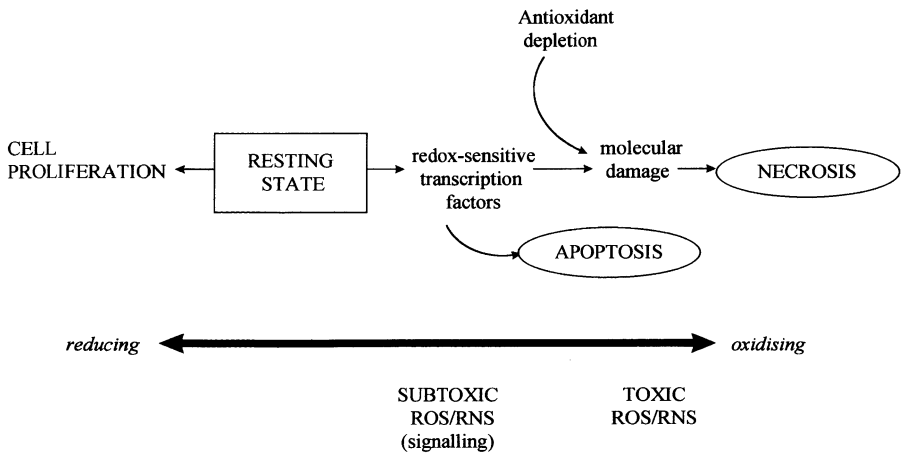

Figure 3. The role of ROS and RNS as second messengers in determining cellular fate are illustrated.

ROS and RNS (for reviews see 139). Transcription factors such as AP-1 [140], and NFκB [141, 142] are redox-sensitive and can be activated by ROS/RNS, apoptosis may ultimately be regulated by mechanisms such as these (see Fig. 3).

5. Concluding Remarks

The direct measurement of oxidants and the detection of specific markers of oxidative damage in both acute and chronic lung injury, are suggestive of the production of ROS and RNS in these disease states. However, in humans the role of these species as contributors or consequential agents to disease processes still remains to be elucidated. In animal models the evidence is more obvious as both oxidants and antioxidants have been shown to exhibit profound and opposing effects in the lung. Much recent interest has centred on the contribution of ROS and RNS, at sublethal levels, to lung function and heart disease. It is now apparent that at low levels, many reactive species can act as second messenger molecules and may be involved in many regulatory steps that determine cellular fate. Understanding these redox regulatory processes may lead to a better understanding of the role of ROS and RNS in lung injury and other disease processes.

6. References

1 Gutteridge JMC (1993) Free radicals in disease processes: a complication of cause and consequence. *Free Rad Res* 19: 141–158
2 Muijsers RBR, Folkerts G, Henricks PAJ, Sadeghi-Hashjin G, NijKamp FP (1997) $ONOO^-$: a two-faced metabolite of nitric oxide. *Life Sci* 60: 1833–1845
3 Bannister WH, Bannister JV (1988) Isolation and characteristation of superoxide dismutase: a personal history and tribute to Joe McCord and Irwin Fridovich. *Free Rad Biol Med* 5: 371–376
4 Halliwell B, Gutteridge JMC (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186: 1–85
5 Beckman JS, Beckman TW, Chan J, Marshall PA, Freeman BA (1990) Apparent hydroxyl radical production by peroxynitrite: Implications for endothelial injury from and superoxide. *Proc Nat Acad Sci* 87: 1620–1624
6 Candeias LP, Patel KB, Stratford MR, Wardman P (1993) Free hydroxyl radicals are formed on reaction between the neutrophil-derived species superoxide anion and hypochlorous acid. *FEBS Letts* 33: 151–153
7 Pryor WA, Squadrito GL (1995) The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. *Am J Physiol* 268: L699–L722
8 Candeias LP, Stratford MR, Wardman P (1994) Formation of hydroxyl radicals on reaction of hypochlorous acid with ferrocyanide, a model iron (II) complex. *Free Rad Res* 20: 241–249
9 Doelman CJ, Bast A (1990) Oxygen radicals in lung pathology. *Free Rad Bio Med* 9: 381–400
10 Hanley N, Kozumbo W, Costa D et al (1993) Induction of DNA single strand breaks in lung cells by ozone exposure *in vivo* and *in vitro*. *Am Rev Respir Dis* 147: A670
11 Cross CE, Motchnik PA, Bruener BA, Jones DA, Kaur H, Ames BN, Halliwell B (1992) Oxidative damage to plasma constituents by ozone. *FEBS Letts* 298: 269–272
12 Weiss SJ (1989) Tissue destruction by neutrophils. *N Engl J Med* 320: 365–376

13 Heinecke JW, Li W, Francis GA, Goldstein JA (1993) Tyrosyl radical generated by myeloperoxidase catalyzes the oxidative cross-linking of proteins. *J Clin Invest* 91: 2866–2872
14 van der Vliet A, Eiserich JP, Halliwell B, Cross CE (1997) Formation of reactive nitrogen species during peroxidase-catalysed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *J Biol Chem* 272: 7617–7625
15 Eiserich JP, Cross CE, Jones AD, Halliwell B, van der Vliet A (1996) Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. A novel mechanism for nitric oxide-mediated modification. *J Biol Chem* 271: 19199–19208
16 Rawls H, Van Santen P (1997) Singlet oxygen: A possible source of the original hydroperoxides in fatty acids. *Ann NY Acad Sci* 171: 135–137
17 Cueto R, Squadrito GL, Bermudez E, Pryor WA (1992) Identification of heptanal and nonenal in bronchoalveolar lavage from rats exposed to low levels of ozone. *Biochem Biophys Res Comm* 188: 129–134
18 Radi R, Beckman JS, Bush KM, Freeman BA (1991) Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 288: 481–487
19 Panasenko OM, Evgina SA, Aidyraliev RK, Sergienko VI, Vladimrov YA (1994) Peroxidation of human blood lipoproteins induced by exogenous hypochlorite or hypochlorite generated in the system of myloperoxidase $^{+}H_2O_2^{+}$ Cl^{-}. *Free Rad Biol Med* 16: 143–148
20 Fukahori M, Ichimori K, Ishida H, Nakagawa H, Okino H (1994) Nitric oxide reversibly suppresses xanthine oxidase activity. *Free Rad Res* 21: 203–212
21 Brooks EC, Mahr NN, Radisavljevic Z, Jacobson ED, Terada LS (1997) Attenuates and xanthine oxidase exaggerates lung damage-induced gut injury. *Am J Physiol* 272: G845–G852
22 Strand V, Rak S, Svartengren M, Bylin G (1997) Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. *Am J Respir Crit Care Med* 155: 881–887
23 Beckman J, Tsai J-H (1994) Reactions and diffusion of nitric oxide and peroxynitrite. *The Biochemist* Oct/Nov 8–10
24 Blough N, Zafiriou O (1985) Reaction of superoxide with nitric oxide to form peroxynitrite in alkaline aqueous solutions. *Inorg Chem* 24: 3502–3504
25 Xia Y, Dawson VL, Dawson TM, Snyder SH, Zweier JL (1996) Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. *Proc Natl Acad Sci USA* 93: 6770–6774
26 Radi R, Beckman JS, Bush K, Freeman BA (1991) Peroxynitrite oxidation of sulfhydryls: The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 266: 4244–4250
27 Gatti RM, Radi R, Augusto O (1994) Peroxynitrite-mediated oxidation of albumin to the protein-thiyl free radical. *FEBS Letts* 348: 287–290
28 Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD, Beckman JS (1992) Peroxynitrite mediated tyrosine nitration catalysed by superoxide dismutase. *Arch Biochem Biophys* 298: 431–437
29 Rubbo AB, Barnes KM, Freeman BA, Radi R (1996) Peroxynitrite-dependent tryptophan nitration. *Chem Res Toxicol* 9: 390–396
30 Butler AR, Rhodes P (1997) Chemistry, analysis, and biological roles of S-nitrosothiols. *Anal Biochem* 249: 1–9
31 Singh RJ, Hogg N, Joseph J, Kalyanaraman B (1996) Mechanism of nitric oxide release from S-nitrosothiols. *J Biol Chem* 271: 18596–18603
32 Davidson CA, Kaminski PM, Wolin MS (1997) NO elicits prolonged relaxation of bovine pulmonary arteries via endogenous peroxynitrite generation. *Am J Physiol* 273: L437–L444
33 Guidot DM, Repine MJ, Hybertson BM, Repine JE (1995) Inhaled nitric oxide prevents neutrophil-mediated, oxygen radical-dependent leak in isolated rat lungs. *Am J Physiol* 269: L2–L5
34 Gutierrez HH, Nieves B, Chumley P, Rivera A, Freeman BA (1996) Nitric oxide regulation of superoxide-dependent lung injury: oxidant-protective actions of endogenously produced and exogenously administered nitric oxide. *Free Rad Biol Med* 21: 43–52
35 Nossuli TO, Reid H, Scalia R, Lefer AM (1997) Peroxynitrite reduces myocardial infarct size and preserves coronary endothelium after ischemia and reperfusion in cats. *Circulation* 96: 2317–2324

36 Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L et al (1994) The American-European consensus on ARDS. *Am J Respir Crit Care Med* 149: 818–824
37 Pittet JF, Mackersie RC, Martin TR, Matthay MA (1997) Biological markers of acute lung injury: prognostic and pathogenic significance. *Am J Respir Crit Care Med* 155: 1187–1205
38 Gerschman R, Gilbert D, Nye S et al (1954) Oxygen poisioning and x-radiation: A mechanism in common. *Science* 199: 623–626
39 Jackson R (1990) Molecular, pharmacologic, and clinical aspects of oxygen induced lung injury. *Clin Chest Med* 1: 73–86
40 Cross CE, Van der Viliet A, O'Neill C, Eiserich J (1994) Reactive oxygen species and the lung. *Lancet* 344: 930–933
41 Deneke S, Fanburg B (1980) Normobaric oxygen toxicity of the lung. *N Engl J Med* 303: 76–86
42 Balaan M, Bowman L, Dedhia H, Miles P (1995) Hyperoxia-induced alterations of rat alveolar lavage composition and properties. *Exp Lung Res* 21: 141–156
43 Clerch L, Massaro D (1993) Tolerance of rats to hyperoxia: Lung antioxidant enzyme gene expression. *J Clin Invest* 91: 499–508
44 Frank L, Summerville J, Massaro D (1980) Protection from oxygen toxicity with endotoxin. Role of the endogenous antioxidant enzymes of the lung. *J Clin Invest* 65: 1104–1110
45 White C, Ghezzi P, Dinarello C, Caldwell S, McMurty I, Repine J (1987) Recombinant tumour necrosis factor/cachectin and interleukin 1 pretreatment decreases lung oxidised glutathione accumulation, lung injury and mortality in rats exposed to hyperoxia. *J Clin Invest* 79: 1868–1873
46 Erzurum S, Danel C, Gillssen A, Chu CS, Trapnell BC, Crystal RG (1993) *In vivo* antioxidant gene expression in human airway epithelium of normal individuals exposed to 100% oxygen. *J Appl Physiol* 75: 1256–1262
47 Fleming R, Whitman I, Gitlin J (1991) Induction of caeruloplasmin gene expression in rat lung during inflammation and hyperoxia. *Am J Physiol* 260: L68–L74
48 Town I, Phillips G, Murdoch E, Holgate S, Kelly F (1993) Temporal association between pulmonary inflammation and antioxidant induction following hyperoxic exposure of the preterm guinea-pig. *Free Rad Res Communs* 18: 211–223
49 Nozik-Grayck E, Piantadosi CA, van Adelsberg J, Alsper SL, Huang YC (1997) Protection of perfused lung from oxidant injury by inhibitors of anion exchange. *Am J Physiol* 273: L296–L304
50 McCord J (1985) Oxygen-derived free radicals in postischaemic tissue injury. *N Engl J Med* 312: 159–163
51 Ferrari R (1994) Oxygen free radicals at myocardial level: effects of ischaemia and reperfusion. In: Armstrong D (ed). Free radicals in diagnostic medicine. A systems approach to laboratory technology, clinical correlations, and antioxidant therapy. New York and London: Plenum Press, 99–122
52 Granger D, Hollwarth M, Parks D (1986) Ischaemia-reperfusion injury: Role of oxygen derived free radicals. *Physiol Scand Suppl* 548: 47–63
53 Ward A, McBurney A, Lunec J (1994) Evidence for the involvement of oxygen-derived free radicals in ischaemia-reperfusion injury. *Free Rad Res* 20: 21–28
54 Parks D, Granger N (1986) Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol Scand* 54: 87–99
55 Wakabayashi Y, Fujita H, Morita I, Kawagushi H, Murota S (1995) Conversion of xanthine oxidase in bovine carotid artery endothelial cells induced by activated neutrophils: involvement of adhesion molecules. *Biochim Biophys Acta* 1265: 103–109
56 Xia Y, Zweier JL (1995) Substrate control of free radical generation from xanthine oxidase in the postischemic heart. *J Biol Chem* 270: 18797–18803
57 Quinlan GJ, Lamb NJ, Tilly R, Evans TW, Gutteridge JMC (1997) Plasma hypoxanthine levels in ARDS: implications for oxidative stress, morbidity and mortality. *Am J Respir Crit Care Med* 155: 479–484
58 Grum C, Ragsdale R, Ketani L, Simon R (1987) Plasma xanthine oxidase activity in patients with adult respiratory distress syndrome. *J Crit Care* 2: 22–26
59 Tate R, Vanbenthuysen K, Shasby D, McMurtry IF, Repine JE (1982) Oxygen radical mediated permeability oedema and vasoconstriction in isolated perfused rabbit lungs. *Am Rev Respir Dis* 126: 802–806

60 Johnson K, Fantone J, Kaplan J, Ward PA (1981) *In vivo* damage of rat lungs by oxygen metabolits. *J Clin Invest* 67: 983–993
61 Faggioni R, Gatti S, Demitri M, Delgado R, Echtenacher B, Gnocchi P et al (1994) Role of xanthine oxidase and reactive oxygen intermediates in LPS and TNF-induced pulmonary oedema. *J Lab Clin Med* 123: 394–399
62 Kurosaki M, Li Calzi M, Scanziani E, Garattini E, Terao M (1995) Tissue- and cell-specific expression of mouse xanthine oxidoreductase gene *in vivo*: Regulation by bacterial LPS. *Biochem J* 306: 225–234
63 Sarnesto A, Linder N, Raivio KO (1996) Organ distribution and molecular forms of human xanthine dehydrogenase/xanthine oxidase protein. *Lab Invest* 74: 48–56
64 Adachi T, Fukushima T, Usami Y, Hirano K (1993) Binding of human xanthine oxidase to sulphated glycosaminoglycans on the endothelial-cell surface. *Biochem J* 289: 523–527
65 Nielsen VG, Tan S, Weinbroum A, McCammon AT, Samuelson PN, Gelman S et al (1996) Lung injury after hepatoenteric Ischemia-reperfusion injury: role of xanthine oxidase. *Am J Respir Crit Care Med* 154: 1364–1369
66 Phan S, Gannon D, Varani J, Ryan U, Ward P (1989) Xanthine oxidase activity in rat pulmonary artery endothelial cells and its alteration by activated neutrophils. *Am J Pathol* 134: 1201–1211
67 Zweier J, Kuppusamy P, Thompson-Gorman S, Klunk D, Lutty G (1994) Measurement and characterisation of free radical generation in reoxygenated human endothelial cells. *Am J Physiol* 266: C700–C708
68 Ward P (1991) Mechanisms of endothelial cell injury. *J Lab Clin Med* 118: 421–426
69 Till G, Friedl H, Ward P (1991) Lung injury and complement activation: Role of neutrophils and xanthine oxidase. *Free Rad Biol Med* 10: 379–386
70 Moores H, Beehler C, Hanley M, Shanley PF, Stevens EE, Repine JE et al (1994) Xanthine oxidase promotes neutrophil sequestration but not injury in hyperoxic lungs. *J Appl Physiol* 76: 941–945
71 Shenkar R, Abraham E (1996) Plasma from hemorrhaged mice activates CREB and increases cytokine expression in lung mononuclear cells through a xanthine oxidase-dependent mechanism. *Am J Respir Cell Mol Biol* 14: 198–206
72 Shenkar R, Schwartz MD, Terada LS, Repine JE, McCord J, Abraham E (1996) Hemorrage activates NK-kappa B in murine lung mononuclear cells *in vivo*. *Am J Physiol* 270: L729–L735
73 Babior B (1984) The respiratory burst of phagocytes. *J Clin Invest* 73: 599–601
74 Thannickal VJ, Fanburg BL (1995) Activation of H_2O_2 – generating NADH oxidase in human lung fibroblasts by transforming growth factor *beta* 1. *J Biol Chem* 270: 30334–30338
75 Kelly F, Rickett G, Philips G (1992) Magnitude of hyperoxic stress and degree of lung maturity determine the nature of pulmonary antioxidant response in the guinea-pig. *Free Rad Res Communs* 17: 335–347
76 Wizemann T, Laskin D (1994) Enhanced phagocytosis, chemotaxis, and production of reactive oxygen intermediates by interstitial lung macrophages following acute endotoxemia. *Am J Respir Cell Mol Biol* 11: 358–365
77 Wang W, Suzuki Y, Tanigaki T, Rank D, Raffin T (1994) Effect of the NADPH oxidase inhibitor apocynin on septic lung injury in guinea-pigs. *Am J Respir Crit Care Med* 150: 1449–1452
78 Wyman M, Tscharner V, Deranleau D, Baggiolini M (1987) Chemiluminescence detection of hydrogen peroxide produced by human neutrophils during the respiratory burst. *Anal Biochem* 165: 371–378
79 Baldwin S, Simon R, Grum C, Ketai LH, Boxer LA, Devall LJ et al (1986) Oxidant activity in expired breath of patients with adult respiratory distress syndrome. Lancet 1: 11–14
80 Sznajder J, Fraiman A, Hall J, Sanders SW, Schmidt G, Crawford G et al (1989) Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. *Chest* 96: 602–612
81 Mathru M, Rooney M, Dries D, Hirsch LJ, Barnes L, Tobin MJ et al (1994) Urine hydrogen peroxide during adult respiratory distress syndrome in patients with and without sepsis. *Chest* 105: 232–236
82 Sibille Y, Reynolds H (1990) Macrophages and polymorphonuclear neutrophils in lung defence and injury. *Am Rev Respir Dis* 141: 471–501

83 Gutteridge JMC, Mumby S, Quinlan GJ, Chung KF, Evans TW (1996) Pro-oxidant iron is present in human pulmonary epithelial lining fluid: implications for oxidative stress in the lung. *Biochem Biophys Res Communs* 220: 1024–1027
84 Lamb NJ, Gutteridge JMC, Baker CS, Evans TW, Quinlan GJ (1999) Oxidative damage to proteins of bronchoalveolar lavage fluid protein in patients with ARDS. Evidence for neutrophil mediated hydroxylation, nitration and chlorination. *Crit Care Med* in press
85 Demling H, Lalonde C, Jin L, Ryan P, Fox R (1986) Endotoximia causes increased lung tissue lipid peroxidation in unanesthetized sheep. *J Appl Physiol* 60: 2094–2100
86 Ward PA, Till GO, Hatherill JR, Annesley TM, Kunkel R (1985) systemic complement activation, lung injury, and the products of lipid peroxidation. *J Clin Invest* 76: 517–527
87 Richard C, Lemonnier F, Thibault M, Couturier M, Auzepy P (1990) Vitamin E deficiency and lipperoxidation during adult respiratory syndrome. *Crit Care Med* 18: 4–9
88 Lefevre G, Brunet F, Bonneau C et al (1994) Human polymorphonuclear leukocyte metabolism and lipopreoxidation during adult respiratory distress syndrome treated by extracorporeal carbon dioxide removal. *Pathophysiology* 1: 13–19
89 Baouali AB, Aube H, Maupoil V, Blettery B, Rochette L (1994) Plasma lipid peroxidation in critically ill patients: importance of mechanical ventilation. *Free Rad Biol Med* 16: 223–227
90 Esterbauer H, Schauer R, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Rad Biol Med* 11: 81–128
91 Schaur RJ, Dussing G, Kink E, Schauenstein E, Posch W, Kukovetz E et al (1994) The lipid peroxidation product 4-hydroxy-2-nonenal is formed by – and is able to attract – rat neutrophils *in vivo*. *Free Rad Res* 20: 365–373
92 Quinlan GJ, Lamb NJ, Evans TW, Gutteridge JMC (1996) Plasma fatty acid changes and increased lipid peroxidation in patients with adult respiratory distress syndrome. *Crit Care Med* 24: 241–246
93 Olafsdottir K, Ryrfeldt A, Atzori L, Berggren M, Moldeus P (1991) Hydroperoxide-induced broncho- and vasoconstriction in the isolated rat lung. *Exp Lung Res* 17: 615–627
94 Pacifici E, Mcleod L, Peterson H, Sevanian A (1994) Linoleic acid hydroperoxide-induced peroxidation of endothelial cell phospholipids and cytotoxicity. *Free Rad Biol Med* 17: 285–295
95 Pacifici E, Mcleod L, Sevanian A (1994) Lipid hydroperoxide-induced peroxidation and turnover of endothelial phospholipids. *Free Rad Biol Med* 17: 297–309
96 Vissers CM, Thomas C (1997) Hypochlorous acid disrupts the adhesive properties of subendothelial matrix. *Free Rad Biol Med* 23: 401–411
97 Merritt A, Amirkanian J, Helbock H, Halliwell B, Cross C (1993) Reduction of the surface-tension-lowering ability of surfactant after exposure to hypochlorous acid. *Biochem J* 295: 19–22
98 Ischiropoulos H, Zhu L, Chen J, Tsai M, Martin JC, Smith CD et al (1992) Peroxynitrite mediated tyrosine nitration catalysed by superoxide dismutase. *Arch Biochem Biophys* 298: 431–437
99 Denicola A, Freeman BA, Trujillo M, Radi R (1996) Peroxynitirte reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys* 333: 49–58
100 Pryor WA, Lemercier JN, Zhang H, Uppu RM, Squadrito GL (1997) The catalytic role of carbon dioxide in the decomposition of peroxynitrite. *Free Rad Biol Med* 23: 331–338
101 Ischiropoulos H, Zhu L, Beckman J (1992) Peroxynitirte formation from activated rat alveolar macrophages. *Arch Biochem Biophys* 298: 446–451
102 Carreras M, Pargament G, Catz S, Poderoso J, Boveris A (1994) Kinetics of nitric oxide and hydrogen peroxide production and formation of peroxynitrite during the respiratory burst of human neutrophils. *FEBS Letts* 341: 65–68
103 Kooy N, Royall J (1994) Antagonist-Induced peroxynitrite production by endothelial cells. *Arch Biochem Biophys* 310: 353–359
104 Kooy N, Royall J, Ye Y, Kelly D, Beckman J (1995) Evidence for *in vivo* peroxynitrite production in human acute lung injury. *Am J Respir Crit Care Med* 151: 1250–1254
105 Radi R, Beckman J, Bush K, Freeman B (1991) Peroxynitrite-induced membrane lipid peroxidation: The cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 288: 481–487

106 Radi R, Beckman J, Bush K, Freeman B (1991) Peroxynitrite oxidation of sulfhydryls: The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 266: 4244–4250
107 Haddad IY, Zhu S, Ischiropoulos H, Matalon S (1996) Nitration of surfactant protein A results in decreased ability to aggregate lipids. *Am J Physiol* 270: L281–L288
108 Zhu S, Haddad IY, Matalon S (1996) Nitration of surfactant protein A (SP-A) tyrosine residues results in decreased mannose binding ability. *Arch Biochem Biophys* 333: 282–290
109 Hu P, Ischiropoulos H, Beckman JS, Matalon S (1994) Peroxynitrite inhibition of oxygen consumption and sodium transport in alveolar type II cells. *Am J Physiol* 266: L628–L634
110 Haddad IY, Zhu S, Crow J, Barefield E, Gadilhe T, Matalon S (1996) Inhibition of alveolar type II cell ATP and surfactant synthesis by nitric oxide. *Am J Physiol* 270: L898–L906
111 Matalon S, DeMarco V, Haddad IY, Myles C, Skimming JW, Schurch S et al (1996) Inhaled nitric oxide injures the pulmonary surfactant systems of lambs *in vivo*. *Am J Physiol* 270: L273–L280
112 Kresek-Staples J, Kew R, Webster R (1992) Caeruloplasmin and transferrin levels are altered in serum and bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 145: 1009–1015
113 Gutteridge JMC, Quinlan GJ, Mumby S, Heath A, Evans TW (1994) Primary plasma antioxidants in adult respiratory distress syndrome patients: Changes in iron-oxidising, iron-binding, and free radical-scavenging proteins. *J Lab Clin Med* 124: 263–273
114 Gutteridge JMC, Quinlan GJ, Evans TW (1994) Transient iron-overload with bleomycin-detectable iron in the plasma of patients with adult respiratory distress syndrome. *Thorax* 49: 707–710
115 Lykens M, Davis B, Pacht E (1992) Antioxidant activity of bronchoalveolar lavage fluid in the adult respiratory distress syndrome. *Am J Physiol* 262: L169–L175
116 Conelly KG, Moss M, Parsons PE, Moore EE, Moore FA, Giclas PC et al (1997) Serum ferritin as a predictor of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 155: 21–25
117 O'Neill C, Halliwell B, van der Vliet A, Davis PA, Packer L, Tritschler H et al (1994) Aldehyde-induced protein modifications in human plasma: Protection by glutathione and dihydrolipoic acid. *J Lab Clin Med* 124: 359–370
118 Bunnell E, Pacht ER (1993) Oxidised glutathione is increased in the alveolar fluid of patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 148: 1174–1178
119 Quinlan GJ, Evans TW, Gutteridge JMC (1994) Oxidative damage to plasma proteins in adult respiratory distress syndrome. *Free Rad Res* 20: 289–298
120 Cross C, Forte T, Stocker R, Louie S, Yamamoto Y, Ames BN et al (1990) Oxidative stress and abnormal cholesterol metabolism in patients with adult respiratory distress syndrome. *J Lab Clin Med* 115: 396–404
121 Leff J, Parson P, Day C, Taniguchi N, Jochum M, Fritz H et al (1993) Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis. *Lancet* 341: 777–780
122 Bernard GR, Wheeler AP, Arons MM, Moris PE, Paz HL, Russell JA et al (1997) A trial of antioxidants N-acetylcysteine and procysteine in ARDS. The antioxidant in ARDS study group. *Chest* 112: 164–172
123 Dekhuijzen PN, Aben KK, Dekker I, Aarts LP, Wielders PL, van Herwaarden CL et al (1996) Increased exhalation of hydrogen peroxide in patients with stable and unstable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 154: 813–816
124 Calhoun WJ, Bush RK (1990) Enhanced reactive oxygen species metabolism of airspace cells and airway inflammation follow antigen challenge in human astham. *J Allergy Clin Immunol* 86: 306–313
125 Repine JE, Bast A, Lankhorst I (1997) Oxidative stress in chronic obstructive pulmonary disease. Oxidative Stress Study Group. *Am J Respir Crit Care Med* 156: 341–357
126 Pinamonti S, Muzzoli M, Chicca MC, Papi A, Ravenna F, Fabbri LM et al (1996) Xanthine oxidase activity in bronchoalveolar lavage fludi from patients with chronic obstructive pulmonary disease. *Free Radic Biol Med* 21: 147–155
127 Smith LJ, Shamsuddin M, Sporn PH, Denenberg M, Anderson J (1997) Reduced superoxide dismutase in lung cells of patients with astham. *Free Radic Biol Med* 22: 1301–1307

128 Kadrabova J, Mad'aric A, Kovacikova Z, Podivinsky F, Ginter E, Gazdik F (1996) Selenium status is decreased in patients with intrinsic asthma. *Biol Trace Elem Res* 52: 241–248
129 Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A et al (1993) Induction of nitric oxide synthase in asthma. *Lancet* 342: 8886–8887
130 Li XY, Gilmour PS, Donaldson K, MacNee W (1996) Free radical activity and proinflammatory effects of particulate air pollution (PM10) *in vivo* and *in vitro*. *Thorax* 51: 1216–1222
131 Brown RK, Wyatt H, Price JF, Kelly FJ (1996) Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress. *Eur Respir J* 9: 334–339
132 Brown RK, McBurney A, Lunec J, Kelly FJ (1995) Oxidative damage to DNA in patients with cystic fibrosis. *Free Radic Biol Med* 18: 801–806
133 Saleh D, Barnes PJ, Giaid A (1997) Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 155: 1763–1769
134 Ali S, Jain SK, Abdulla M, Athar M (1996) Paraquat induced DNA damage by reactive oxygen species. *Biochem Mol Biol Int* 39: 63–67
135 Sarafian TA, Bredesen DE (1994) Is apoptosis mediated by reactive oxygen species? *Free Radic Res* 21: 1–8
136 Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Valentine JS et al (1993) Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262: 1274–1277
137 Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Corsmeyer SJ (1993) Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75: 241–251
138 Lennon SV, Martin SJ, Cotter TG (1991) Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell Prolif* 24: 203–214
139 Sen CK, Packer L (1996) Antioxidant and redox regulation of gene transcription. *FASEB J* 10: 709–720
140 Abate C, Patel L, Rauscher FJ, Curran T (1990) Redox regulation of fos and jun DNA-binding activity *in vitro*. *Science* 249: 1157–1161
141 Staal FJ, Roederer M, Herzenberg LA, Herzenberg LA (1990) Intracellular thiols regulate activation of nuclear factor kappa B and transcription of human immunodeficiency virus. *Proc Natl Acad Sci* 87: 9943–9947
142 Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κ B transcription factor and HIV-1. *EMBO J* 10: 2247–2258